

FORM PTO-1350U S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 5-93)		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		3477-88
		U.S. APPLICATION NO. (If known see 37 C.F.R. 1.5) 09/600358
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA99/00038	18 January 1999	16 January 1998
TITLE OF INVENTION		
HUMAN LYMPHOID PROTEIN TYROSINE PHOSPHATASES		
APPLICANT(S) FOR DO/EO/US		
Chaim M. ROIFMAN		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(I). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> has been transmitted by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). 7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 		
Items 11. to 16. below concern other document(s) or information included:		
<ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. <input checked="" type="checkbox"/> Other items or information: PCT Request; International Search Report; International Preliminary Examination Report; PCT Demand; Sequence Listing (Paper and Computer copy); Verified Statement Claiming Small Entity Status 		

U.S. APPLICATION NO (If known, see 37 C.F.R. 1.50) <div style="font-size: 1.5em; font-weight: bold;">09/600358</div>		INTERNATIONAL APPLICATION NO PCT/CA99/00038		ATTORNEY'S DOCKET NUMBER 3477-88	
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17. <input checked="" type="checkbox"/> The following fees are submitted: <div style="margin-left: 20px;"> Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO \$840.00 International preliminary examination fee paid to USPTO (37 CFR 1.482). \$670.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$760.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO. \$970.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4). \$96.00 ENTER APPROPRIATE BASIC FEE AMOUNT = \$840.00 </div>	CALCULATIONS	PTO USE ONLY																				
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	\$																					
<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <th style="width:20%;">Claims</th> <th style="width:20%;">Number Filed</th> <th style="width:20%;">Number Extra</th> <th style="width:20%;">Rate</th> </tr> <tr> <td>Total Claims</td> <td>39 - 20 =</td> <td>19</td> <td>X \$18.00</td> </tr> <tr> <td>Independent Claims</td> <td>25 - 3 =</td> <td>22</td> <td>X \$78.00</td> </tr> <tr> <td colspan="3">Multiple dependent claim(s) (if applicable)</td> <td>+ \$260.00</td> </tr> <tr> <td colspan="3">TOTAL OF ABOVE CALCULATIONS =</td> <td>\$2,898.00</td> </tr> </table>	Claims	Number Filed	Number Extra	Rate	Total Claims	39 - 20 =	19	X \$18.00	Independent Claims	25 - 3 =	22	X \$78.00	Multiple dependent claim(s) (if applicable)			+ \$260.00	TOTAL OF ABOVE CALCULATIONS =			\$2,898.00	\$ 342.00 \$1,716.00 \$	
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Total Claims	39 - 20 =	19	X \$18.00																			
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Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).	\$1,449.00																					
SUBTOTAL =	\$1,449.00																					
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	\$																					
TOTAL NATIONAL FEE =	\$1,449.00																					
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +	\$																					
TOTAL FEES ENCLOSED =	\$1,449.00																					
	Amount to be refunded	\$																				
	charged	\$																				

a. ☒ A check in the amount of **\$1,449.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the
 above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
 overpayment to Deposit Account No. 50-0220.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must
 be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

Karen A. Magri
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 Post Office Box 37428
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"Express Mail" mailing label number EL481791325US
 Date of Deposit: July 14, 2000

I hereby certify that this paper or fee is being deposited
 with the United States Postal Service "Express Mail Post
 Office to Addressee" service under 37 CFR 1.10 on the
 date indicated above and is addressed to Box PCT,
 Commissioner for Patents, Washington, DC 20231.

Margaret J. Pfeiffer
 Margaret J. Pfeiffer
 Date of Signature: July 14, 2000

Karen Magri

SIGNATURE

41,965
 REGISTRATION NUMBER

534 Rec'd PCT/PTO 14 JUL 2000
PATENT

PATENT

In re: Application of Roifman
Serial No.: To be Assigned
Filed: Concurrently Herewith
For: *HUMAN LYMPHOID PROTEIN
TRYOSINE PHOSPHATASES*

BOX PCT
Commissioner for Patents
Washington, DC 20231

Sir:

In the Specification.

After the title, please insert the following:

--Related Application Information

This application claims the benefit under 35 U.S.C. § 371 from PCT Application No. PCT/CA99/00038, filed January 18, 1999, the disclosure of which is incorporated by reference herein in its entirety, which claims the benefit of Canadian Application Serial No. 2,220,853, filed January 16, 1998, the disclosure of which is incorporated by reference herein in its entirety.--

In the Claims.

Please amend the claims as follows.

11. (Amended) A recombinant vector comprising a polynucleotide of [any of claims] claim 1 [to 10].

[illegible]

Marjorie J. Pfeiffer
Date of Signature: July 14, 2000

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27 (d)) - NONPROFIT ORGANIZATION

Docket No.

Serial No.

PCT/CA99/00038

Filing Date

January 18, 1999

Patent No.

Issue Date

Applicant/ Chaim M. Roifman

Patentee:

Invention: **HUMAN LYMPHOID PROTEIN TYROSINE PHOSPHATASES**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: HSC Research and Development Limited Partnership

ADDRESS OF ORGANIZATION: Suite 5270, 555 University Avenue

Toronto, Ontario M5G 1X8

Canada

TYPE OF NONPROFIT ORGANIZATION:

- ☐ University or other Institute of Higher Education
- ☐ Tax Exempt under Internal Revenue Service Code (26 U.S.C. 501(a) and 501(c)(3))
- ☐ Nonprofit Scientific or Educational under Statute of State of The United States of America
Name of State: Citation of Statute:
- ☒ Would Qualify as Tax Exempt under Internal Revenue Service Code (26 U.S.C. 501(a) and 501(c)(3)) if Located in The United States of America
- ☐ Would Qualify as Nonprofit Scientific or Educational under Statute of State of The United States of America if Located in The United States of America
Name of State: Citation of Statute:

I hereby declare that the above-identified nonprofit organization qualifies as a nonprofit organization as defined in 37 C.F.R. 1.9(e) for purposes of paying reduced fees to the United States Patent and Trademark Office regarding the invention described in:

- ☐ the specification to be filed herewith.
- ☒ the application identified above.
- ☐ the patent identified above.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the above-identified nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed on the next page and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- ☒ no such person, concern or organization exists.
☐ each such person, concern or organization is listed below.

FULL NAME

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Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING:

ANNE MARIE CHRISTIAN

TITLE IN ORGANIZATION:

PRESIDENT

ADDRESS OF PERSON SIGNING:

SIGNATURE:



DATE:

July 13/08

Human Lymphoid Protein Tyrosine Phosphatases

534 Rec'd PCT/PTC 14 JUL 2000

Field of the Invention

This invention relates to a human phosphatase gene (*Lyp*) which encodes an intracellular tyrosine phosphatase (Lyp1) and an isoform of Lyp1, called Lyp2. More particularly, it relates to the cDNA sequence of human Lyp1 and Lyp2, the protein products and the expression, role and use of these phosphatases in humans.

Background of the Invention

Protein tyrosine phosphorylation, a key mechanism of cellular signal transduction, is regulated by the action of both protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases). Originally PTKs were believed to control the process of tyrosine phosphorylation, with a small number of PTPases playing largely housekeeping roles. Unexpectedly, the structural diversity of the growing number of PTPases has called this idea into question and it has become apparent that PTPases have important roles in the regulation of growth and differentiation in both normal and neoplastic cells (1,2).

All PTPases contain a catalytic domain of approximately 200-300 residues including a subset of highly conserved amino acids that play a role in substrate recognition and tyrosine dephosphorylation (3). The PTPases family can be divided broadly into two major classes: membrane bound, receptor-like or receptor phosphatases and non-receptor or intracellular phosphatases (4, 5). Both types can be further subdivided into subfamilies based on their sequence similarities and non-catalytic domain structure motifs (6,7).

The receptor PTPases have one or two intracellular phosphatase domains and often have Ig-like domains and fibronectin-like extracellular regions (6) that play a role in cell-cell or cell-matrix interactions (8). In fact, some receptor PTPases appear to participate in homophilic and heterophilic binding interactions suggestive of a role in cell guidance and contact inhibition (7,8).

The non-receptor phosphatases display various intracellular localizations determined by amino acid sequences outside the catalytic domain (9, 35, 40). Some contain conserved non-catalytic domains such as the Src homology 2 (SH2) and SH3

domains allowing them to interact with a variety of tyrosine phosphorylated proteins and proteins containing proline rich sequences (19, 10, 11). Cytoplasmic PTPases have been found associated with a variety of PTKs including CSK and the Jak kinases, and a number of cytokine and antigen receptors (7, 11,31).

Several lines of evidence indicate that within the immune system, PTPases are essential for lymphocyte development and activation. CD45, a transmembrane phosphatase expressed exclusively in hematopoietic cells (12), is required for antigenic activation of B and T lymphocytes (13, 14). In addition, evidence from CD45-deficient mice indicates that CD45 also plays a pivotal role in thymic development and T cell apoptotic response to T cell receptor engagement (15, 16). Recent studies have suggested that the hematopoietic-specific intracellular phosphatase-SHP1 (SH2 containing PTPase) negatively regulates signaling through association with the B cell receptor, PcyRIIB1 (17) and the IL-3 receptor 3 chain (18). SHP1 also participates in T cell signalling events through dephosphorylation of the T cell receptor (TCR), p56^{lck} and ZAP-70 (19). Mutations in the murine *motheaten* locus coding the SHP1 protein result in severe combined immunodeficiency and systemic autoimmunity, as well as many other hematopoietic abnormalities (20). Furthermore, expression of HePTP, a cytoplasmic hematopoietic-specific PTPase, is induced in lymphocytes stimulated by phytohemagglutinin, concavalin A, lipopolysaccharide and anti-CD3 (21), suggestive of a role in lymphocyte activation pathways.

Summary of the Invention

A human gene encoding a novel cytoplasmic phosphatase protein, Lyp1, has been identified and designated Lymphoid Protein Tyrosine Phosphatase gene (*Lyp*). The *Lyp* gene has been localized to human chromosome 1p13.

The phosphatase Lyp1 is involved in lymphocyte growth and development and is predominantly expressed in lymphoid cells. Lyp1 is an intracellular 105 kDa protein containing a single tyrosine phosphatase catalytic domain.

In addition, an isoform of Lyp1 has been identified and designated Lyp2. This isoform is a product of C-terminal alternative RNA splicing resulting in a smaller 85 kDa protein.

The cDNA sequences encoding Lyp1 and Lyp2 have been cloned, sequenced and expressed to provide the respective proteins. The proteins are most highly expressed in lymphoid tissues including spleen, lymph nodes, peripheral leukocytes, tonsil, bone marrow, thymocytes and in both immature and mature B and T lymphocytes. Lyp1 expression has been demonstrated to be induced by activation of resting peripheral T lymphocytes with PHA or anti-CD3. Lyp1 has also been demonstrated to be constitutively associated with the proto-oncogene c-Cbl (a protein which is recognized to be important in the regulation of the Zap family kinases) in thymocytes and T cells. Overexpression of Lyp1 reduced Cbl tyrosine phosphorylation, suggesting it may be a substrate of the phosphatase. Lyp1 has also been demonstrated to down-regulate the activity of the T cell tyrosine kinase Zap-70, with little effect on Syk kinase. Lyp1 strongly down-regulates Lyn kinase activity, while Fyn function is unaffected. In B cells, Lyp is constitutively bound to the Syk kinase and inducibly binds a number of phosphorylated proteins after stimulation of the cell through the B cell receptor.

It therefore appears that the protein products of the Lyp gene are important for regulation of T cell antigen receptor signalling and cytokine receptor signalling.

In accordance with one embodiment, the invention provides an isolated polynucleotide comprising a nucleotide sequence encoding a Lyp protein.

In accordance with a further embodiment, the invention provides an isolated polynucleotide which encodes a Lyp protein having an amino acid sequence of greater than 70% overall identity to the amino acid sequence of Table 2.

In accordance with a further embodiment, the invention provides an isolated polynucleotide which encodes a Lyp protein having an amino acid sequence of greater than 70% overall identity to the amino acid sequence of Table 4.

In accordance with a further embodiment, the invention provides an isolated polynucleotide which encodes a Lyp protein having an amino acid sequence with at least 80% overall identity, preferably at least 90% overall identity to the amino acid sequence of Table 2.

In accordance with a further embodiment, the invention provides an isolated polynucleotide which encodes a Lyp protein having an amino acid sequence with at

least 80% overall identity, preferably at least 90% overall identity to the amino acid sequence of Table 4.

In accordance with a further embodiment, the invention provides a nucleotide sequence comprising at least 10, preferably 15 and more preferably 20 consecutive nucleotides of Sequence ID NO:1 or Sequence ID NO:3.

In accordance with a further embodiment, the invention provides a substantially purified Lyp protein.

In accordance with a further embodiment, the invention provides a substantially purified protein having an amino acid sequence of greater than 70% overall identity to the amino acid sequence of Table 2.

In accordance with a further embodiment, the invention provides a substantially purified protein having an amino acid sequence of greater than 70% overall identity to the amino acid sequence of Table 4.

In accordance with a further embodiment, the invention provides a substantially purified protein having an amino acid sequence with at least 80% overall identity, preferably at least 90% overall identity, to the amino acid sequence of Table 2.

In accordance with a further embodiment, the invention provides a substantially purified protein having an amino acid sequence with at least 80% overall identity, preferably at least 90% overall identity, to the amino acid sequence of Table 4.

In accordance with a further embodiment, the invention provides a peptide comprising at least 5, preferably 10, more preferably 20 consecutive amino acids of Sequence ID NO:2 or Sequence ID NO:4.

In accordance with a further embodiment, the invention provides a peptide comprising at least one functional domain of a Lyp protein.

In accordance with a further embodiment, the invention provides a peptide comprising at least one antigenic determinant of a Lyp protein.

In accordance with a further embodiment, the invention provides an antibody which binds specifically to a Lyp protein.

In accordance with a further embodiment, the invention provides a method for screening a candidate compound for an ability to increase or decrease the phosphatase activity of a Lyp protein comprising

- (a) providing an assay system for assaying Lyp phosphatase activity;
- (b) assaying Lyp phosphatase activity in the presence or absence of the candidate compound; and
- (c) determining whether the Lyp phosphatase activity was higher or lower in the presence of the candidate compound than in its absence.

In accordance with a further embodiment, the invention provides a method for screening a candidate compound for ability to modulate expression of a Lyp gene comprising

contacting a cell with a candidate compound, wherein the cell includes a regulatory region of a Lyp gene operably joined to a coding region; and
detecting a change in expression of the coding region.

In accordance with a further embodiment, the invention provides a non-human animal wherein a genome of said animal, or of an ancestor thereof, has been modified by a modification selected from the group consisting of:

- (a) knockout of a Lyp gene; and
- (b) insertion of a polynucleotide encoding a heterologous Lyp gene.

In accordance with a further embodiment, the invention provides a pharmaceutical composition comprising an active ingredient selected from the group consisting of:

- (a) an isolated nucleotide sequence encoding a Lyp protein;
- (b) a substantially purified Lyp protein;
- (c) a substantially purified antibody which binds specifically to a Lyp protein

and a pharmaceutically acceptable carrier.

In accordance with a further embodiment, the invention provides a method for treating a subject having a deficiency of Lyp activity comprising administering to the subject an effective amount of an agent selected from the group consisting of:

- (a) an isolated nucleotide sequence encoding a Lyp protein;
- (b) a substantially purified Lyp protein.

In accordance with a further embodiment of the invention, a method is provided for identifying allelic variants or heterospecific homologues of a Lyp1 gene comprising

- (a) choosing a nucleic acid probe or primer capable of hybridizing to a human Lyp1 gene sequence under stringent hybridization conditions;
- (b) mixing said probe or primer with a sample of nucleic acids which may contain a nucleic acid corresponding to the variant or homologue;
- (c) detecting hybridization of the probe or primer to the nucleic acid corresponding to the variant of homologue.

In accordance with a further embodiment of the invention, a pharmaceutical composition is provided comprising an active ingredient selected from the group consisting of:

- an antisense sequence which hybridizes to a human Lyp1 nucleotide sequence or to a transcript of the sequence;
- a substantially pure antibody which binds selectively to human Lyp1 or Lyp2 protein and a pharmaceutically acceptable carrier;
- a mimetic of human Lyp1 or Lyp2 protein;
- a functional analog of human Lyp1 or Lyp2 protein;
- an inhibitor of human Lyp1 or Lyp2 protein activity; and
- an agent capable of altering the phosphorylation state of human Lyp1 or Lyp2 protein.

In accordance with a further embodiment of the invention, a method is provided of screening for an agent useful in treating a disorder characterized by an abnormality in a phosphorylation signaling pathway of lymphoid cells, wherein the pathway involves an interaction between a human Lyp1 or Lyp2 protein and a human Lyp1 or Lyp2 activator, comprising screening potential agents for ability to disrupt or promote the interaction as an indication of a useful agent.

In accordance with a further aspect of the invention, a method is provided of preventing or treating a disorder in a mammal characterized by an abnormality in an intracellular phosphorylation signaling pathway of lymphoid cells, wherein the pathway involves an interaction between a human Lyp1 or Lyp2 protein and a human

Lyp1 or Lyp2 substrate, comprising the step of disrupting or promoting said interaction *in vivo*.

Brief Description of the Drawings

Certain embodiments of the invention are described, reference being made to the accompanying drawings, wherein:

Figure 1 shows a schematic diagram of Lyp1 and Lyp2 deduced from the cDNA clones. Boxes indicate the open reading frame, with thin lines representing the 5' and 3' untranslated regions. The six overlapping cDNA clones (bold black lines) obtained from a human thymus cDNA library are shown under the schematic structures of the cDNAs.

Figure 2 shows an alignment of Lyp1 and Z70PEP (Sequence ID NO:5) amino acid sequences. The PTPase domain is indicated by brackets. An arrow indicates the end of the amino acid sequence shared by Lyp1 and Lyp2 and the beginning of the unique C-terminal sequence of Lyp1. The NXXY motif is indicated by line above the sequence. The four potential SH3 domain binding sites are also indicated (asterisks). A consensus sequence is shown below the alignment. The unique seven amino acids of Lyp2 are shown in the box below the alignment.

Figure 3A (left panel) demonstrates the regional mapping of the Lyp gene by fluorescence *in situ* hybridization to normal human lymphocyte chromosomes counterstained with DAPI. Biotinylated cDNA probe was detected with avidin-fluorescein isothiocyanate (FITC). Separate images of DAPI counterstained metaphase chromosomes and of Lyp cDNA probe hybridization signals were captured and overlaid electronically. Part of a representative metaphase preparation is shown to indicate the position of the Lyp probe FISH signals that are visible as two yellow fluorescent spots on the p arm of chromosome 1. Figure 3A, right panel, shows a DAPI banded chromosome. Figure 3B shows a schematic ideogram of the DAPI banded chromosome of Figure 3A, right panel, indicating that the Lyp1 probe hybridizes to band 1p13.

Figure 4 demonstrates that Lyp2 is a result of alternative splicing of the Lyp gene. A, schematic map of the PCR strategy used. Primer 1 corresponds to the last 20 nucleotides shared by both Lyp1 and Lyp2 sequences, primer 2 to Lyp2 untranslated area and primer 3 to the beginning of the novel Lyp1 sequence,

immediately downstream of primer 1. (see also C). B. The results of the PCT amplification on genomic DNA. Lane 1, DNA size markers, Lane 2, a product of 3.5kb was amplified with primers 1 and 3. Lane 3, is a product of 100bp was amplified with primers 1 and 2. C. Schematic map of Lyp1 splicing. The sequences before the vertical line represent the splice donor site, while the nucleotide sequences after it are the Lyp1 intronic sequence which code for the unique C-terminal seven amino acids, stop codon (asterisk) and untranslated sequence (lower case letters) of Lyp1. A white box represents the common cDNA sequence shared by Lyp1 and Lyp2, the black and the light gray boxes representing the unique sequences of each cDNA (Lyp1 and Lyp2 respectively).

Figure 5 shows the expression profile of Lyp1 and Lyp2 transcripts. The size of RNA markers are indicated in kb. A. Human tissues of various origin. B/C. Immune relevant human tissues.

Figure 6 shows an immunoblot of transfected COS-7 cells. T7 tagged Lyp1 (A) or Lyp2 (B) were transfected into COS-7 cells and immunoprecipitated with anti-Lyp or anti-T7 antibody and blotted with anti-T7. (A): Lyp1 transfection results in a transfected protein of 105 kD and a probable degradation product of 96 kD, while (B): shows Lyp2 as a protein of 85 kD.

Figure 7 shows *in vivo* translation of Lyp1 and Lyp2 proteins, COS-7 cells were transiently transfected with HA-tagged Lyp1 and Lyp2 cDNAs and protein expression analyzed by Western blotting with anti-HA tag antibodies. Lane 1, pCDNA3. Lane 2, pCDNA3 Lyp1. Lane 3, pCDNA3 Lyp2. Molecular mass markers are shown in kDa.

Figure 8 shows the relative quantification of Lyp1 and Lyp2 transcripts in thymocytes cDNA by competitive PCR. Different concentration of competitor DNA were added to fixed amount of sample cDNA. The results of PCR amplification products of (A) 26 cycles with specific primers to Lyp1 (B) 35 cycles with specific primers to Lyp2. The internal control concentrations are indicated below the pictures in Pico-Molar.

Figure 9 shows the localization of Lyp1 and Lyp2 in transiently transfected COS-7 cells by immunofluorescence. COS-7 cells were transiently transfected with HA-tagged Lyp1 and Lyp2 cDNAs and immunofluorescence was performed using a

monoclonal antibody against HA tag. Magnification 1000X. (A) Cells transfected with HA-Lyp2 cDNA. (B) Cells transfected with HA-Lyp1 cDNA.

Figure 10 shows Lyp protein expression in lymphoid and myeloid cell lines. Lyp was immunoprecipitated from cell lines (10^7 cells) and blotted with Lyp antibodies. A protein band of 105 kD corresponding to Lyp1 could be detected in Jurkat, Daudi, Ramos, A1 and G2 cells, while U937 and K562 do not appear to have detectable amounts of Lyp. (PB)- Pre-immune serum control.

Figure 11 shows the expression of Lyp proteins in resting and activated T cells. A. Lyp was precipitated from thymocytes (80×10^6 cells), peripheral blood T cells (25×10^6 cells) and tonsil T cells (10×10^6 cells) and immunoblotted with anti-Lyp. Pre-immune serum controls (PB) are presented in each case. A band of 105 kD is present in each sample and a band of 85 kD can be seen only in resting peripheral T cells. B. Lyp was immunoprecipitated from peripheral blood T-cells (25×10^6 cells) before and after stimulation with anti-CD3 (2.5 $\mu\text{g/ml}$) or PHA over a period of 48 hours. There is increased 105 kD Lyp1 expression, while the 85 kD protein appears to be down regulated.

Figure 12 shows the measurement of Lyp1 phosphatase activity. Anti-Lyp immunoprecipitates from untransfected and pcDNA3-Lyp1 transfected cells were prepared in pervanadate free lysis buffer and incubated with ^{33}P labelled substrate Raytide. At the indicated time points reactions were stopped by the addition of charcoal and the free ^{33}P released from the peptide and now present in the supernatant measured by liquid scintillation counting.

Figure 13 demonstrates the involvement of Lyp1 in TCR signaling. (A) Lyp immunoprecipitates from thymocytes (80×10^6 cells) stimulated with anti-CD3 were blotted with antiphosphotyrosine. A single phosphorylated band of 116 kD was detected co-immunoprecipitating with Lyp. Lyp protein loading was quantitated by anti-Lyp western blot after stripping. (B) Immunoblotting with anti-Cbl identified the 116 kD phosphorylated protein as Cbl, while immunoblotting with anti-FAK or anti-p110 (subunit of PI-3 kinase) showed them not to be associated with Lyp. (C) Lyp1 was transfected into COS-7 cells and Cbl immunoprecipitates prepared from these and untransfected cells. Western blotting was performed with Lyp antibodies. The

position of Lyp is indicted by an arrow. Cbl immunoprecipitates were also prepared and blotted with anti-phosphotyrosine (D), and then anti-Cbl after stripping.

Figure 14 shows the immunoprecipitation of the Lyp1, ZAP-70 and FYN proteins from (a) COS-7 monkey epithelial cell line or (B) the 293T human epithelial cell line, the cell lines being transfected with the cDNA for Lyp1, ZAP-70 or Fyn in the eucaryotic expression vector pcDNA3. Western blotting was performed with anti-phosphotyrosine antibodies and chemiluminescent detection reagents. In both cell lines, Lyp1 co-transfection resulted in a reduction in Zap-70 phosphorylation while Fyn was unaffected. Lyp1 down-regulated Zap-70 after activation by Fyn in 293-T cells (B), lanes 3 and 4. Syk was unaffected by Lyp1 (C).

Figure 15 shows shows the immunoprecipitation of the Lyp1, Jak3, Syk and Fyn proteins from COS-7 monkey epithelial cell line, the cell line being transfected with the cDNA for Lyp1, Jak3, Syk, or Fyn in the eucaryotic expression vector pcDNA3. Syk was also co-transfected with Lyp-N, a catalytically inactive form of Lyp1. Western blotting was performed with anti-phosphotyrosine antibodies and chemiluminescent detection reagents. Lyp1 reduced the tyrosine phosphorylation of Jak3 (C) while having little effect on Syk (D). No effect was seen when Syk was co-transfected with Lyp-N (D).

In the drawings, preferred embodiments of the invention are illustrated by way of example. It is to be expressly understood that the description and drawings are for the purpose of illustration and as an aid to understanding, and are not intended as a definition of the limits of the invention.

Description of the Invention

A novel intracellular human phosphatase gene, *Lyp*, has been isolated and identified. Lyp is predominantly expressed in the lymphoid cell lineages. Lyp is a member of the PEST phosphatase family and is most closely related to the murine phosphatase Z70PEP. Hydropathy analysis has indicated that Lyp contains no obvious signal sequence or hydrophobic segments and thus apparently encodes a cytoplasmic protein containing a single catalytic phosphatase domain. The non-catalytic portions of the phosphatase contain areas of high proline, glutamic acid, serine and threonine content (PEST sequences). There also appear to be other

formally recognized functional domain structures within the remainder of the protein. A short linear amino acid sequence also found in PEP has been demonstrated to bind the murine phosphatase to the cytoplasmic tyrosine kinase csk. There are otherwise several areas of high proline content which potentially may be recognized by SH3 domains.

Two forms of messenger RNA have been isolated for Lyp. The longer encodes the entire protein of 808 amino acids, Lyp1, while the second shorter form arises from alternative splicing of the RNA and encodes Lyp2, which has 692 amino acids.

A 3.5kb intronic sequence of Lyp1 was found to contain an alternative exon, coding for the C-terminal 7 amino acids of Lyp2, and at least part of its 3' untranslated area (Fig.4). The Lyp2 coding sequence consequently reads into this intronic sequence until a termination codon is encountered. As a result, the two proteins have the first 685 amino acids in common, and the catalytic domain of the two forms is identical, as is most of the non-catalytic area. However, the final 123 amino acids of Lyp1 are absent in Lyp2 and are replaced by seven unique residues. This is highly suggestive of major differences in the regulation of the activity of the two isoforms. Studies of expression of the proteins suggests that Lyp2 may only be present in resting lymphoid cells, while Lyp1 expression is increased upon activation.

Two novel intracellular protein tyrosine phosphatase cDNA sequences have been isolated from a human thymus cDNA library; the first for Lyp1 (GenBank Accession No. AF 001846) and the second for its splice variant, Lyp2 (GenBank Accession No. AF001847). The cDNA sequences for Lyp1 and Lyp2 (Sequence ID Nos:1 and 3) are shown in Tables 1 and 3, respectively. The corresponding amino acid sequences for Lyp1 and Lyp2 (Sequence ID Nos:2 and 4) are shown in Tables 2 and 4 respectively.

Sequence analysis of Lyp1 reveals significant homology with the murine phosphatase Z70PEP, an intracellular PTPase widely expressed in hematopoietic tissues (10). Lyp1 shares an overall amino acid sequence identity of 70% with Z70PEP (Fig. 2). While there is 89% identity between the catalytic domain of Lyp1 and Z70PEP, significantly less homology is observed within the non catalytic portion (61%), which clearly contains a large area of unique sequence. Within this low

homology area, Lyp1 contains four proline rich sequences which are also present in PEP (Fig. 2), forming putative PXXP and class II (XPPLPXR) SH3 domain binding motifs (10, 31). It has been demonstrated that an association exists between one of the proline-rich motifs of PEP (PPPLPERTP, also present in Lyp) and the SH3 domain of the protein tyrosine kinase p50csk (32). Experiments also show Lyp1 to associate with csk in T cells (data not shown). The Lyp1 non-catalytic domain also contains a large area of unique sequence, including an NXXY motif (Fig. 2). When tyrosine phosphorylated, this motif may be recognized by a phosphotyrosine binding (PTB) domain (29) found in adaptor proteins such as IRS, Shc and cbl.

The murine Z70PEP also possesses several consensus PEST sequences (hence its name [PEST]-domain Phosphatase)(10). PEST sequences contain an unusually high percentage of proline (P), glutamic/aspartic acid (E/D), serine (S), and threonine(T) residues. An analysis of the Lyp1 sequence using the program PEST-FIND (PC analysis software; Oxford Molecular Group, Oxford) indicated the presence of only a single PEST region (amino acids 702-736), while five were confirmed in Z70PEP.

Through immunofluorescent staining of transiently transfected Cos-7 cells, it was determined that both Lyp1 and Lyp2 show a similar pattern of diffuse cytoplasmic staining (Fig. 9).

The significance of the alternative C-terminal sequences of Lyp1 and Lyp2 remains unclear, but there are several differences between the C-terminal tails that may be key in revealing functional divergence. The C-terminus of Lyp1, but not Lyp2, contains a consensus sequence XS/TPXK/R (⁷⁴¹KTPGK⁷⁴⁵) recognized by the p34^{cdc2} kinase (41), a cell cycle regulatory kinase (42), suggesting that Lyp1 may be phosphorylated in a cell cycle dependent manner. Lyp1 also contains four potential SH3 domain binding sites, compared to a single motif in Lyp2; suggesting the isoforms may interact with different sets of SH3 domains.

The pattern of Lyp1 expression observed by Northern blotting suggests that it is preferentially expressed in lymphoid cells (Fig. 5A,B,C), particularly in thymocytes and mature B and T cells. A low level of Lyp1 expression was also seen in tissues rich in lymphoid infiltrates, such as the small intestine and appendix. The pattern of Lyp1 protein expression detected by antibodies in human hematopoietic cell lines

correlated well with Lyp1 mRNA expression (Fig. 11). This pattern of expression suggests that Lyp1 may play a role in the regulation of aspects of both early and late states of T cell differentiation. The lack of expression in fetal liver tissue, which contains a large population of pre-B cells, may suggest a different role in the biology of B cell development. The mRNA expression of Lyp1 and its isoform, Lyp2, was differentiated by the use of more specific probes. While Lyp2 was present at lower levels than Lyp1 in all lymphoid tissues examined, Northern blot analysis indicated significant expression of Lyp2 in fetal liver tissue. Lymphoid mRNAs hybridized with a probe specific for the unique C-terminal of Lyp1 revealed the same pattern of expression seen in Northern blots obtained by using a cDNA fragment common to both Lyp forms (not shown). This suggests that Lyp2 expression in lymphoid cells is extremely low, below the threshold of detection of Northern blotting. This suggestion was confirmed by semi-quantitative PCR comparison of Lyp1 and Lyp2 expression. In thymocytes, the expression of Lyp1 was found to be 100 fold greater than that of Lyp2 (Fig. 8). Similar results were obtained from other lymphoid cells (not shown).

Resting peripheral T lymphocytes demonstrated expression of an 85 kD protein recognized by the Lyp specific antibodies. Stimulation of T lymphocytes with PHA or anti-CD3 resulted in the induction of the Lyp1 protein, with a simultaneous down regulation of the 85 kD protein (Fig. 11). The 85 kD protein is believed to be Lyp2 on the basis of its apparent molecular weight and the fact that both Lyp antibodies can recognize it. This finding suggests that Lyp2 may play an important role in resting cells, since thymocytes, tonsil T cells and lymphoid cell lines, which are activated cells, do not express the protein.

Anti-CD3 stimulation of thymocytes was found to induce the association of a 116 kD phosphorylated protein with Lyp1. Western blotting of Lyp immunoprecipitates identified the phosphorylated band to be the proto-oncogene c-Cbl. Although inducibly phosphorylated, cbl was found to be constitutively associated with Lyp1. From previous studies it is known that Cbl is heavily tyrosine phosphorylated following TCR stimulation (58) and can associate with the Syk and ZAP tyrosine kinases, negatively regulating their activities (59-63). Treatment of Jurkat cells with the phosphatase inhibitor pervanadate leads to a marked increase in the phosphorylation of Cbl (61) suggesting that tyrosine phosphatases keep Cbl in a

basally dephosphorylated state. It has been now demonstrated that Lyp1 is basally associated with Cbl in thymocytes; this interaction was confirmed in Jurkat cells (data not shown) and in COS cells by transfection, where Cbl phosphorylation was also reduced by Lyp1 overexpression (Fig. 13). This strongly suggests that Lyp may play a role in regulating Cbl activity through modulation of its tyrosine phosphorylation status. As Cbl is an adaptor protein which associates with numerous protein tyrosine kinases, it is possible that Lyp may play a role in the regulation of these proteins (62). Although direct tyrosine phosphorylation of Lyp1 was not detected, a minor variant (EPNY) of the Cbl PTB domain consensus binding motif (D(N/D)XpY) is present in the non-catalytic domain, which could form the basis for interaction. Alternatively, in the absence of other identifiable interactive domains in either protein, a multiple SH3 domain adaptor protein such as Grb2 may serve to link Lyp and Cbl.

Thus it appears that Lyp1 is constitutively associated in T cells with the proto-oncogene c-Cbl, a protein which is recognized to be important in the regulation of the Zap family kinases. In B cells, Lyp is constitutively bound to the Syk kinase and inducibly binds a number of phosphorylated proteins after stimulation of the cell through the B cell receptor.

It has also been demonstrated that Lyp1 reduces phosphorylation of, and thereby reduces the activity of, the T cell tyrosine kinase, Zap-70, while it has little effect upon the closely related Syk kinase, possibly even elevating its activity. A similar selective activity is seen with members of the src family of kinases. Lyp1 strongly down-regulates Lyn kinase activity, while Fyn function is unaffected. The ability to turn off Zap-70 and Lyn strongly suggests a role for Lyp in regulating antigen receptor signalling, as these kinases are central to the signal transduction cascades. Overexpression of Lyp1 in T cells appears to interfere with activation of the Zap-70 kinase through the T cell receptor.

Lyp1 also reduced phosphorylation of, and thereby reduced the activity of, the cytoplasmic Jak3 tyrosine kinase and prevented it from phosphorylating the insulin receptor substrate proteins. Thus, Lyp is also in a position to regulate signal transduction through a number of the cytokine receptors. Activation of Jak kinases appears to be a primary event after ligand binding to cytokine receptors and absolutely necessary for signal transduction.

Co-transfection of COS cells with encoding sequences for Lyp1 and Zap70 kinase, or for Lyp1 and Jak3 kinase, led to complete dephosphorylation of Zap70 and of Jak3, suggesting that Lyp1 regulates Zap70 and Jak3 activities in T cells. Co-transfection with Lyp1 and Syk kinase, a relative of Zap70, showed no Syk dephosphorylation, indicating a selective effect of Lyp1 on Zap70 and Jak3. This selectivity was further demonstrated by experiments performed by including Lyp1 and src family kinases, such as lck and fyn, which again showed no dephosphorylation.

T lymphocytes lacking functional Zap 70 protein do not respond satisfactorily to antigenic stimulation and since antigenic stimulation is required for normal T cell maturation, such lymphocytes fail to mature properly (Arpaia et al., (1994), Cell, v. 76, pp. 947-958; Perlmutter, R., (1994), Nature, v. 370, p. 249). The importance of Zap 70 kinase in T cell antigen receptor signalling also means that if one can interfere with or prevent Zap 70 kinase activity, one can modulate T cell activation and proliferation in situations where T cell activation and proliferation is excessive or undesired. Stimulators or activators of Lyp1 could be used as drugs which, by reducing Zap70 activity, could reduce or block T cell activation.

Similarly, over-expression of Lyp1 could be used to control T cell activation.

The proliferation of T cells also depends on IL2 receptor signal transduction, which involves Jak3 kinase activation. Hence increasing Lyp1 activity will reduce or prevent T cell proliferation by reducing Jak3 kinase activity.

Over-expression of, or stimulation of the activity of, Lyp1 therefore provides a two-locus control of T cell activation and proliferation, (1) by blocking initial signals transmitted via the T cell receptor and (2) by blocking progression of T cell proliferation by blocking IL2-mediated responses.

This selective effect of Lyp1 renders it an ideal target for candidate immunosuppressive drugs which can be used, for example, in organ or tissue graft rejection, graft versus host disease, and autoimmune diseases, including diabetes, rheumatic diseases, multiple sclerosis and other nervous system diseases.

Furthermore, Jak3 kinase activity is of crucial importance in the proliferation of lymphoma cells. Reducing or blocking the activity of Jak3 kinase and Zap70 kinase by manipulating the activity of Lyp1, for example by causing its over-

expression, provides a powerful means of reducing or preventing the growth of T cell lymphomas.

Similarly, Zap70 and Jak3 kinase activities are important in thymocyte differentiation and control of these kinase activities by manipulation of Lyp1 activity provides a method for controlling thymocyte differentiation.

Using FISH analysis, the Lyp gene was found to be localized to chromosome 1p13 (Fig. 3). This region is of particular interest because it is a common site of chromosomal rearrangement in both solid and hematopoietic cancers (47, 48). One such chromosomal rearrangement is a frequent alteration in the 1p13 region in chromosomally aberrant clones isolated from both patients with Hodgkin's (49, 50) and non Hodgkin's (51) lymphomas. Several lines of evidence already suggest that PTPases may act as tumour suppression genes (2, 52). This suggests an association between an abnormality of the 1p13 locus in these patients and an alteration of Lyp and thus an involvement of Lyp in tumorigenesis.

Isolated Nucleic Acids

In accordance with one series of embodiments, this invention provides isolated polynucleotides corresponding to the nucleotide sequences encoding the human Lyp1 and Lyp2 proteins. The polynucleotides may be in the form of DNA, genomic DNA, cDNA or mRNA or an anti-sense DNA corresponding to a disclosed sequence. Also provided are portions of the Lyp sequences useful as probes and PCR primers or for encoding fragments, functional domains or antigenic determinants of Lyp proteins.

One of ordinary skill in the art is now enabled to identify and isolate Lyp genes or cDNAs which are allelic variants of the disclosed Lyp sequences, using standard hybridization screening or PCR techniques.

Depending on the intended use, the invention provides portions of the disclosed nucleotide sequences comprising about 10 consecutive nucleotides (e.g. for use as PCR primers) to nearly the complete disclosed nucleotide sequences. The invention provides isolated nucleotide sequences comprising sequences corresponding to at least 10, preferably 15 and more preferably at least 20 consecutive nucleotides of the Lyp gene as disclosed or enabled herein or their complements.

In addition, the isolated polynucleotides of the invention include any of the above described nucleotide sequences included in a vector.

In accordance with a further embodiment, the invention provides an isolated polynucleotide which encodes a Lyp protein having an amino acid sequence of greater than 70% overall identity to the amino acid sequence of Table 2 or 4.

The invention also includes polynucleotides which are complementary to the disclosed nucleotide sequences, polynucleotides which hybridize to these sequences under high stringency and degeneracy equivalents of these sequences.

In accordance with a further embodiment, the invention also includes an isolated polynucleotide which encodes a Lyp protein having an amino acid sequence with at least 80% overall identity, preferably at least 90% overall identity to the amino acid sequence of Table 2 or 4.

Proteins

This invention provides Lyp proteins and a method for producing such proteins.

In accordance with one embodiment, a Lyp protein has an amino acid sequence having greater than 70% overall identity to the amino acid sequence of Lyp1 (Table 2, Sequence ID No:2).

In accordance with a further embodiment, a Lyp protein has an amino acid sequence having at least 80% overall identity, preferably at least 90%, to the amino acid sequence of Lyp1.

In accordance with a further embodiment, the invention provides substantially purified Lyp proteins, including the proteins of Table 2 (Lyp1) and Table 4 (Lyp2).

The invention includes analogs of the disclosed protein sequences, having conservative amino acid substitutions therein. The invention also includes fragments of the disclosed protein sequences, such as peptides comprising at least 5, preferably 10 and more preferably 20 consecutive amino acids of the disclosed protein sequences.

The invention further provides polypeptides comprising at least one functional domain or at least one antigenic determinant of a Lyp protein.

In accordance with a further embodiment, the invention enables the production

of Lyp proteins, such as human Lyp 1 and Lyp 2.

Lyp proteins may be produced by culturing a host cell transformed with a DNA sequence encoding a selected Lyp protein. The DNA sequence is operatively linked to an expression control sequence in a recombinant vector so that the protein may be expressed.

Host cells which may be transfected with the vectors of the invention may be selected from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus subtilis*, or other bacilli, yeasts, fungi, insect cells or mammalian cells including human cells.

For transformation of a mammalian cell for expression of a Lyp protein, the vector may be delivered to the cells by a suitable vehicle. Such vehicles including vaccinia virus, adenovirus, retrovirus, Herpes simplex virus and other vector systems known to those of skill in the art.

A Lyp protein may also be recombinantly expressed as a fusion protein. For example, the Lyp cDNA sequence is inserted into a vector which contains a nucleotide sequence encoding another peptide (e.g. GST-glutathione succinyl transferase). The fusion protein is expressed and recovered from prokaryotic (e.g. bacterial or baculovirus) or eukaryotic cells. The fusion protein can then be purified by affinity chromatography based upon the fusion vector sequence and the Lyp protein obtained by enzymatic cleavage of the fusion protein.

The protein may also be produced by conventional chemical synthetic methods, as understood by those skilled in the art.

Lyp proteins may also be isolated from cells or tissues, including mammalian cells or tissues, in which the protein is normally expressed.

The protein may be purified by conventional purification methods known to those in the art, such as chromatography methods, high performance liquid chromatography methods or precipitation.

For example, an anti-Lyp antibody may be used to isolate a Lyp protein which is then purified by standard methods.

To produce Lyp proteins recombinantly, for example *E. coli* can be used using the T7 RNA polymerase/promoter system using two plasmids or by labeling of plasmid-encoded proteins, or by expression by infection with M13 Phage mGPI-2. *E. coli* vectors can also be used with Phage lambda regulatory sequences, by fusion

protein vectors (eg. lacZ and trpE), by maltose-binding protein fusions, and by glutathione-S-transferase fusion proteins.

Alternatively, the Lyp1 or Lyp2 protein can be expressed in insect cells using baculoviral vectors, or in mammalian cells using vaccinia virus. For expression in mammalian cells, the cDNA sequence may be ligated to heterologous promoters, such as the simian virus (SV40) promoter in the pSV2 vector and introduced into cells, such as COS cells to achieve transient or long-term expression. The stable integration of the chimeric gene construct may be maintained in mammalian cells by biochemical selection, such as neomycin and mycophenolic acid.

The Lyp DNA sequence can be altered using procedures such as restriction enzyme digestion, fill-in with DNA polymerase, deletion by exonuclease, extension by terminal deoxynucleotide transferase, ligation of synthetic or cloned DNA sequences, site-directed sequence alteration with the use of specific oligonucleotides together with PCR.

The cDNA sequence or portions thereof, or a mini gene consisting of a cDNA with an intron and its own promoter, is introduced into eukaryotic expression vectors by conventional techniques. These vectors permit the transcription of the cDNA in eukaryotic cells by providing regulatory sequences that initiate and enhance the transcription of the cDNA and ensure its proper splicing and polyadenylation. The endogenous Lyp gene promoter can also be used. Different promoters within vectors have different activities which alters the level of expression of the cDNA. In addition, certain promoters can also modulate function such as the glucocorticoid-responsive promoter from the mouse mammary tumor virus.

Some of the vectors listed contain selectable markers or neo bacterial genes that permit isolation of cells by chemical selection. Stable long-term vectors can be maintained in cells as episomal, freely replicating entities by using regulatory elements of viruses. Cell lines can also be produced which have integrated the vector into the genomic DNA. In this manner, the gene product is produced on a continuous basis.

Vectors are introduced into recipient cells by various methods including calcium phosphate, strontium phosphate, electroporation, lipofection, DEAE dextran, microinjection, or by protoplast fusion. Alternatively, the cDNA can be introduced by

infection using viral vectors.

Eukaryotic expression systems can be used for many studies of the Lyp gene and gene product(s) including determination of proper expression and post-translational modifications for full biological activity, identifying regulatory elements located in the 5' region of the Lyp gene and their role in tissue regulation of protein expression, production of large amounts of the normal and mutant protein for isolation and purification, to use cells expressing the Lyp1 or Lyp2 protein as a functional assay system for antibodies generated against the protein or to test effectiveness of pharmacological agents, or as a component of a signal transduction system, to study the function of the normal complete protein, specific portions of the protein, or of naturally occurring and artificially produced mutant proteins.

Using the techniques mentioned, the expression vectors containing the Lyp1 or Lyp2 cDNA sequence or portions thereof can be introduced into a variety of mammalian cells from other species or into non-mammalian cells.

The recombinant cloning vector, according to this invention, comprises the selected DNA of the DNA sequences of this invention for expression in a suitable host. The DNA is operatively linked in the vector to an expression control sequence in the recombinant DNA molecule so that Lyp1 or Lyp2 protein can be expressed. The expression control sequence may be selected from the group consisting of sequences that control the expression of genes of prokaryotic or eukaryotic cells and their viruses and combinations thereof. The expression control sequence may be selected from the group consisting of the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of the fd coat protein, early and late promoters of SV40, promoters derived from polyoma, adenovirus, retrovirus, baculovirus, simian virus, 3-phosphoglycerate kinase promoter, yeast acid phosphatase promoters, yeast alpha-mating factors and combinations thereof.

Expression of the Lyp gene in heterologous cell systems may also be used to demonstrate structure-function relationships as well as to provide cell lines for the purposes of drug screening. Ligating the Lyp DNA sequence into a plasmid expression vector to transfect cells is a useful method to test the proteins influence on various cellular biochemical parameters including the identification of substrates as

well as activators and inhibitors of the phosphatase. Plasmid expression vectors containing either the entire coding sequence for Lyp1 or Lyp2, or for portions thereof, can be used in *in vitro* mutagenesis experiments which will identify portions of the protein crucial for regulatory function.

The DNA sequence can be manipulated in studies to understand the expression of the gene and its product. The changes in the sequence may or may not alter the expression pattern in terms of relative quantities, tissue-specificity and functional properties.

Antibodies

In accordance with another embodiment, the present invention enables antibodies which bind selectively to a Lyp protein or to an antigenic determinant of a Lyp protein. As used herein with respect to an antibody, an antibody is said to "bind selectively" to a target if the antibody recognises and binds to the target of interest but does not substantially recognise and bind to other molecules in a sample which includes the target of interest.

Generation of antibodies enables the visualization of the protein in cells and tissues using Western blotting. In this technique, proteins are run on polyacrylamide gel and then transferred onto nitrocellulose membranes. These membranes are then incubated in the presence of the antibody (primary), then following washing are incubated to a secondary antibody which is used for detection of the protein-primary antibody complex. Following repeated washing, the entire complex is visualized using colourimetric or chemiluminescent methods.

Antibodies to an Lyp protein also allow for the use of immunocytochemistry and immunofluorescence techniques in which the proteins can be visualized directly in cells and tissues. This is useful to establish the subcellular location of the protein and the tissue specificity of the protein.

Antibodies to an Lyp protein may also be used to inhibit the protein's activity, where reduced activity is desired.

In general, methods for the preparation of antibodies are well known (42). In order to prepare polyclonal antibodies, fusion proteins containing, for example, defined portions or all of the Lyp1 or Lyp2 protein or specific Lyp1 or Lyp2

generated mutants can be synthesized in bacteria by expression of corresponding DNA sequences in a suitable cloning vehicle. The protein can then be purified, coupled to a carrier protein and mixed with Freund's adjuvant (to help stimulate the antigenic response by the rabbits) and injected into rabbits or other laboratory animals. Alternatively, protein can be isolated from cultured cells expressing the protein. Following booster injections at bi-weekly intervals, the rabbits or other laboratory animals are then bled and the sera isolated. The sera can be used directly or purified prior to use, by affinity chromatography. The sera can then be used to probe protein extracts run on a polyacrylamide gel to identify the Lyp1 or Lyp2 protein or mutant protein. Alternatively, synthetic peptides can be made to the antigenic portions of these proteins and used to inoculate the animals.

To produce monoclonal Lyp1 or Lyp2 antibodies, cells actively expressing the protein are cultured or isolated from tissues and the cell extracts isolated. The extracts or recombinant protein extracts, containing the Lyp1 or Lyp2 protein, are injected in Freund's adjuvant into mice. After being injected 9 times over a three week period, the mice spleens are removed and resuspended in phosphate buffered saline (PBS). The spleen cells serve as a source of lymphocytes, some of which are producing antibody of the appropriate specificity. These are then fused with a permanently growing myeloma partner cell, and the products of the fusion are plated into a number of tissue culture wells in the presence of a selective agent such as HAT. The wells are then screened to identify those containing cells making useful antibody by ELISA. These are then freshly plated. After a period of growth, these wells are again screened to identify antibody-producing cells. Several cloning procedures are carried out until over 90% of the wells contain single clones which are positive for antibody production. From this procedure a stable line of clones is established which produce the antibody. The monoclonal antibody can then be purified by affinity chromatography using Protein A Sepharose. Suitable methods for antibody production may be found in standard texts such as Antibody Engineering, 2nd Edition, Barreback, E.D., Oxford University Press (1995).

Transgenic Animal Models

In accordance with a further embodiment, the invention provides non-human

transgenic animals and methods for the production of non-human transgenic animals which afford models for further study of Lyp proteins and tools for screening candidate compounds as potential therapeutic agents. For example, knock-out animals such as mice may be produced with deletion of the Lyp gene. These animals may be examined for phenotypic changes and used to screen candidate compounds for effectiveness to reverse these changes.

In general, techniques of generating transgenic animals are widely accepted and practiced. A laboratory manual on the manipulation of the mouse embryo, for example, is available, detailing standard laboratory techniques for the production of transgenic mice (41).

There are several ways in which to create an animal model in which the Lyp gene is expressed. One could simply generate a specific mutation in the mouse Lyp gene as one strategy. Alternatively, a wild type human Lyp gene and/or a humanized murine gene could be inserted into the animals genome by homologous recombination. It is also possible to insert a mutant (single or multiple) human gene as genomic or minigene construct using wild type or mutant or artificial promoter elements. More commonly, and most preferred in the present invention, knock-out of the endogenous murine genes may be accomplished by the insertion of artificially modified fragments of the endogenous gene by homologous recombination. The modifications include insertion of mutant stop codons, the deletion of DNA sequences, or the inclusion of recombination elements (lox p sites) recognized by enzymes such as Cre recombinase.

In general, for gene knock-out, embryonic stem cells heterozygous for a knockout mutation in a gene of interest (ie. Lyp gene) and homozygous for a marker gene (eg. coat colour) are transplanted into the blastocoel cavity of 4.5 day embryos homozygous for an alternate marker. The early embryos then are implanted into a pseudopregnant female. Some of the resulting progeny are chimeras. Chimeric mice then are backcrossed. Intercrossing will eventually produce individuals homozygous for the disrupted allele that is, knockout mice. (Capecchi, MR. 1989. Science. 244:1299-1291).

To inactivate the Lyp mouse gene, chemical or x-ray mutagenesis of mouse gametes, followed by fertilization, can be applied. Heterozygous offspring can then

be identified by Southern blotting to demonstrate loss of one allele by dosage, or failure to inherit one parental allele using RFLP markers.

To create a transgenic mouse, a mutant or normal version of the human Lyp gene can be inserted into a mouse germ line using standard techniques of oocyte microinjection or transfection or microinjection into stem cells. Alternatively, if it is desired to inactivate or replace the endogenous Lyp gene, homologous recombination using embryonic stem cells may be applied.

For oocyte injection, one or more copies of a mutant or normal Lyp gene can be inserted into the pronucleus of a just-fertilized mouse oocyte. This oocyte is then reimplanted into a pseudo-pregnant foster mother. The liveborn mice can then be screened for integrants using analysis of tail DNA for the presence of human Lyp gene sequences. The transgene can be either a complete genomic sequence injected as a YAC or chromosome fragment, a cDNA with either the natural promoter or a heterologous promoter, or a minigene containing all of the coding region and other elements found to be necessary for optimum expression.

Retroviral infection of early embryos can also be done to insert the human Lyp gene. In this method, the human Lyp gene is inserted into a retroviral vector which is used to directly infect mouse embryos during the early stages of development to generate a chimera, some of which will lead to germline transmission.

Homologous recombination using stem cells allows for the screening of gene transfer cells to identify the rare homologous recombination events. Once identified, these can be used to generate chimeras by injection of mouse blastocysts, and a proportion of the resulting mice will show germline transmission from the recombinant line. This methodology is especially useful if inactivation of the human Lyp gene is desired. For example, inactivation of the Lyp gene can be done by designing a DNA fragment which contains sequences from a Lyp exon flanking a selectable marker. Homologous recombination leads to the insertion of the marker sequences in the middle of an exon, inactivating the Lyp gene. DNA analysis of individual clones can then be used to recognize the homologous recombination events.

Screening for Lyp Mutations

In another embodiment of the invention, the knowledge of the human Lyp

sequence provides for screening for diseases involving abnormally activated or inactivated Lyp1 or Lyp2 in which the activity defect is due to a mutant Lyp gene. For example, unregulated Jak 3 kinase leads to tumorigenesis (Schwaller, J. et al., (1998), EMBO J., v. 17, p. 5321-33; Lacronique et al., (1997), Science, v. 278, p. 1309-12; Peeters et al., (1997), Blood, v. 90, p. 2535-40). A loss of Lyp activity, for example through a null mutation of Lyp, may lead to tumour formation, for example leukemia. Other defects associated with loss of Lyp function may include autoimmune disorders such as rheumatoid arthritis.

People at risk for a lymphoid disease or, individuals not previously known to be at risk, or people in general may be screened routinely using probes to detect the presence of a mutant Lyp gene by a variety of techniques. Genomic DNA used for the diagnosis may be obtained from body cells, such as those present in the blood, tissue biopsy, surgical specimen, or autopsy material. The DNA may be isolated and used directly for detection of a specific sequence or may be PCR amplified prior to analysis. RNA or cDNA may also be used. To detect a specific DNA sequence hybridization using specific oligonucleotides, direct DNA sequencing, restriction enzyme digest, RNase protection, chemical cleavage, and ligase-mediated detection are all methods which can be utilized. Oligonucleotides specific to mutant sequences can be chemically synthesized and labelled radioactively with isotopes, or non-radioactively using biotin tags, and hybridized to individual DNA samples immobilized on membranes or other solid-supports by dot-blot or transfer from gels after electrophoresis. The presence or absence of these mutant sequences is then visualized using methods such as autoradiography, fluorometry, or colorimetric reaction. Suitable PCR primers can be generated which are useful for example in amplifying portions of the subject sequence containing identified mutations.

Direct DNA sequencing reveals sequence differences between normal and mutant Lyp DNA. Cloned DNA segments may be used as probes to detect specific DNA segments. PCR can be used to enhance the sensitivity of this method. PCR is an enzymatic amplification directed by sequence-specific primers, and involves repeated cycles of heat denaturation of the DNA, annealing of the complementary primers and extension of the annealed primer with a DNA polymerase. This results in an exponential increase of the target DNA.

Other nucleotide sequence amplification techniques may be used, such as ligation-mediated PCR, anchored PCR and enzymatic amplification as would be understood by those skilled in the art.

Sequence alterations may also generate fortuitous restriction enzyme recognition sites which are revealed by the use of appropriate enzyme digestion followed by gel-blot hybridization. DNA fragments carrying the site (normal or mutant) are detected by their increase or reduction in size, or by the increase or decrease of corresponding restriction fragment numbers. Genomic DNA samples may also be amplified by PCR prior to treatment with the appropriate restriction enzyme and the fragments of different sizes are visualized under UV light in the presence of ethidium bromide after gel electrophoresis.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. Small deletions may also be detected as changes in the migration pattern of DNA heteroduplexes in non-denaturing gel electrophoresis. Alternatively, a single base substitution mutation may be detected based on differential primer length in PCR. The PCR products of the normal and mutant gene could be differentially detected in acrylamide gels.

Nuclease protection assays (S1 or ligase-mediated) also reveal sequence changes at specific locations. Alternatively, to confirm or detect a polymorphism restriction mapping changes ligated PCR, ASO, REF-SSCP and SSCP may be used. Both REF-SSCP and SSCP are mobility shift assays which are based upon the change in conformation due to mutations.

DNA fragments may also be visualized by methods in which the individual DNA samples are not immobilized on membranes. The probe and target sequences may be in solution or the probe sequence may be immobilized. Autoradiography, radioactive decay, spectrophotometry, and fluorometry may also be used to identify specific individual genotypes.

According to an embodiment of the invention, the portion of the DNA segment that is informative for a mutation can be amplified using PCR. The DNA segment immediately surrounding a specific mutation acquired from peripheral blood

or other tissue samples from an individual can be screened using constructed oligonucleotide primers. This region would then be amplified by PCR, the products separated by electrophoresis, and transferred to membrane. Labelled probes are then hybridized to the DNA fragments and autoradiography performed.

Drug Screening Methods

In accordance with one embodiment, the invention enables a method for screening candidate compounds for their ability to increase or decrease the phosphatase activity of a Lyp protein. The method comprises providing an assay system for assaying Lyp phosphatase activity, assaying the phosphatase activity in the presence or absence of the candidate compound and determining whether the compound has increased or decreased the control phosphatase activity.

The effect of a candidate compound on Lyp phosphatase activity may be determined, for example, in an assay system such as that described in Example 7 herein.

In accordance with a further embodiment, the invention enables a method for screening candidate compounds for their ability to increase or decrease expression of a Lyp protein. The method comprises contacting a cell with a candidate compound, wherein the cell includes a regulatory region of a Lyp gene operably joined to a coding region, and detecting a change in expression of the coding region.

In one embodiment, the present invention enables culture systems in which cell lines which express the Lyp gene, and thus Lyp1 and/or Lyp2 protein products, are incubated with candidate compounds to test their effects on Lyp expression. Such culture systems can be used to identify compounds which upregulate or downregulate Lyp expression or its function through the interaction with other proteins.

Such compounds can be selected from protein compounds, chemicals and various drugs which are added to the culture medium. After a period of incubation in the presence of a selected test compound(s), the expression of Lyp can be examined by quantifying the levels of Lyp mRNA using standard Northern blotting procedure as described in the examples included herein to determine any changes in expression as a result of the test compound. Cell lines transfected with constructs expressing Lyp can also be used to test the function of compounds developed to modify the protein

expression. In addition, transformed cell lines expressing a normal Lyp protein could be mutagenized by the use of mutagenizing agents to produce an altered phenotype in which the role of mutated Lyp can be studied in order to study structure/function relationships of the protein products and their physiological effects.

Alternatively, rather than evaluating the levels of Lyp expression in the presence of a test compound, other proteins which interact with the Lyp protein products may be assessed through phosphorylation assays as are described herein in the examples. Such assays would identify the role of certain compounds on Lyp function and subsequent intracellular protein interaction and physiological effect.

The effect of drugs/agents which interact with the Lyp protein normal function could be studied in order to more precisely define the intracellular role of Lyp1 and Lyp2 proteins with respect to other proteins. In the present invention, it is demonstrated that Lyp1 down-regulates T cell Zap-70 tyrosine kinase activity and thus activation of T cells. Lyp1 is also demonstrated to down-regulate Lyn kinase activity. This strongly suggests a role for Lyp1 in the regulation of antigen receptor signalling. Therefore, these pathways could be further elucidated by the identification of drugs/agents which alter Lyp1 and thus such antigen receptor signalling and further downstream physiological effects. Such cell culture assays may elucidate the specific nature of Lyp1 in the regulation of the Zap and Lyn family kinases. Incubating cell cultures expressing Lyp with agents that affect phosphorylation may also help to elucidate the involvement of other down stream proteins such as DNA-binding proteins and transcription factors in transcription regulation.

As Lyp is demonstrated to down-regulate the activity of the cytoplasmic Jak3 tyrosine kinase and prevent this kinase from phosphorylating the IRS substrate proteins, cell culture assays as described herein can help to identify candidate compounds to inhibit the effect of Lyp on Jak3 tyrosine kinase activity or modify its effect and thus down-stream intracellular signalling and physiological effects. This may help to identify compounds which regulate activation of cytokine receptors which act through the Jak3 tyrosine kinase signal transduction cascade.

All testing for novel drug development is well suited to defined cell culture systems which can be manipulated to express Lyp and study the result of Lyp protein signalling and gene transcription. Animal models are also important for testing novel

drugs and thus may also be used to identify any potentially useful compound affecting Lyp expression and activity and thus physiological function.

Compounds which are found to increase the phosphatase activity of Lyp protein, or to increase expression of Lyp protein, are lead compounds with potential as immunosuppressive agents, for example by reducing or preventing T cell activation. Such immunosuppressive agents can be employed to treat conditions requiring immunosuppression, including autoimmune diseases such as rheumatic diseases, diabetes, and multiple sclerosis and transplant situations, where suppression of graft rejection or graft versus host reactions are required.

Treatment

This invention enables a method for modulating signalling mediated by the T cell receptor, the method comprising administering to a T cell an agent which increases Lyp phosphatase activity or increases Lyp expression in the T cell.

The invention further enables a method for reducing or preventing T cell activation and/or proliferation, the method comprising administering to the T cell an agent which increases Lyp phosphatase activity or increases Lyp expression in the T cell.

The invention further enables a method for treating a disorder which requires immunosuppression, the method comprising administering to the subject in need of treatment an immunosuppression-effective amount of an agent which increases Lyp phosphatase activity or increases Lyp expression.

In accordance with a further embodiment, the invention enables a method for treating lymphoma in a subject, the method comprising administering to the subject an agent which increases Lyp phosphatase activity or increases Lyp expression in an amount effective to reduce or prevent lymphoma cell proliferation.

The invention further provides methods for preventing or treating disorders characterised by an abnormality in the T cell receptor signalling pathway or the IL2-mediated signalling pathway, comprising modulating signalling by administration of an agent which increases or decreases Lyp phosphatase activity or Lyp expression. T cell receptor signalling modulation is useful in disorders such as autoimmune diseases and in transplant situations, as discussed elsewhere herein.

In accordance with another embodiment, the present invention enables gene therapy as a potential therapeutic approach, in which normal copies of the Lyp gene are introduced into patients to successfully code for normal Lyp1 or Lyp2 protein in several different affected cell types. Mutated copies of the Lyp gene in which the protein product is inactivated can also be introduced into patients.

Retroviral vectors can be used for somatic cell gene therapy especially because of their high efficiency of infection and stable integration and expression. The targeted cells however must be able to divide and the expression of the levels of normal protein should be high. The full length Lyp gene can be cloned into a retroviral vector and driven from its endogenous promoter or from the retroviral long terminal repeat or from a promoter specific for the target cell type of interest (such as lymphoid cells).

Other viral vectors which can be used include adeno-associated virus, vaccinia virus, bovine papilloma virus, or a herpesvirus such as Epstein-Barr virus.

Gene transfer could also be achieved using non-viral means requiring infection *in vitro*. This would include calcium phosphate, DEAE dextran, electroporation, and protoplast fusion. Liposomes may also be potentially beneficial for delivery of DNA into a cell. Although these methods are available, many of these are lower efficiency.

Transplantation of normal genes or mutated genes which code for an inactive Lyp1 or Lyp2 into a targeted affected area of the patient can also be useful therapy for any disorder in which Lyp activity is deficient. In this procedure, a Lyp gene is transferred into a cultivatable cell type such as lymphoid cells, either exogenously or endogenously to the patient. The transformed cells are then injected into the patient.

The invention also provides a method for reversing a transformed phenotype resulting from the excess expression of the Lyp human gene sequence, and/or hyperactivation of a Lyp1 or Lyp2 protein product. Antisense based strategies can be employed to explore gene function, inhibit gene function and as a basis for therapeutic drug design. The principle is based on the hypothesis that sequence specific suppression of gene expression can be achieved by intracellular hybridization between mRNA and a complementary anti-sense species. It is possible to synthesize anti-sense strand nucleotides that bind the sense strand of RNA or DNA with a high degree of specificity. The formation of a hybrid RNA duplex may interfere with the processing/transport/translation and/or stability of a target mRNA.

Hybridization is required for an antisense effect to occur. Antisense effects have been described using a variety of approaches including the use of antisense oligonucleotides, injection of antisense RNA, DNA and transfection of antisense RNA expression vectors.

Therapeutic antisense nucleotides can be made as oligonucleotides or expressed nucleotides. Oligonucleotides are short single strands of DNA which are usually 15 to 20 nucleic acid bases long. Expressed nucleotides are made by an expression vector such as an adenoviral, retroviral or plasmid vector. The vector is administered to the cells in culture, or to a patient, whose cells then make the antisense nucleotide. Expression vectors can be designed to produce antisense RNA, which can vary in length from a few dozen bases to several thousand.

Antisense effects can be induced by control (sense) sequences. The extent of phenotypic changes are highly variable. Phenotypic effects induced by antisense are based on changes in criteria such as biological endpoints, protein levels, protein activation measurement and target mRNA levels.

Multidrug resistance is a useful model for the study of molecular events associated with phenotypic changes due to antisense effects since the MDR phenotype can be established by expression of a single gene *mdr1* (MDR gene) encoding P-glycoprotein (a 170 kDa membrane glycoprotein, ATP-dependent efflux pump).

In the present invention, mammalian cells in which the Lyp human cDNA has been transfected and which express an abnormal phenotype, can be additionally transfected with anti-sense Lyp (Lyp1 or Lyp2) nucleotide DNA sequences which hybridize to the Lyp gene in order to inhibit the transcription of the gene and reverse or reduce the abnormal phenotype. Alternatively, portions of the Lyp gene can be targeted with an anti-sense Lyp sequence specific for the kinase domains or the unique amino terminal sequence which may be responsible for the malignant phenotype. Expression vectors can be used as a model for anti-sense gene therapy to target the Lyp which is expressed in abnormal cells. In this manner abnormal cells and tissues can be targeted while allowing healthy cells to survive. This may prove to be an effective treatment for cell abnormalities induced by Lyp1 or Lyp2.

Immunotherapy is also possible for the treatment of diseases associated with excess Lyp activity. Antibodies can be raised to a hyperactive Lyp1 or Lyp2 protein

(or portion thereof) and then be administered to bind or block the abnormal protein and its deleterious effects. Simultaneously, expression of the normal protein product could be encouraged. Administration could be in the form of a one time immunogenic preparation or vaccine immunization. An immunogenic composition may be prepared as injectables, as liquid solutions or emulsions. The Lyp protein may be mixed with pharmaceutically acceptable excipients compatible with the protein. Such excipients may include water, saline, dextrose, glycerol, ethanol and combinations thereof. The immunogenic composition and vaccine may further contain auxiliary substances such as emulsifying agents or adjuvants to enhance effectiveness. Immunogenic compositions and vaccines may be administered parenterally by injection subcutaneously or intramuscularly.

The immunogenic preparations and vaccines are administered in such amount as will be therapeutically effective, protective and immunogenic. Dosage depends on the route of administration and will vary according to the size of the host.

Examples

The examples are described for the purposes of illustration and are not intended to limit the scope of the invention.

Methods of molecular genetics, protein and peptide biochemistry and immunology referred to but not explicitly described in this disclosure and examples are reported in the scientific literature and are well known to those skilled in the art.

Example 1 - Isolation of Novel Human Phosphatases, Lyp1 and Lyp2

Thymuses were obtained from children undergoing open heart surgery. Mononuclear cells were isolated by Ficoll-Hypaque gradient. Adherent cells were removed by incubation to plastic dishes for 60 minutes at 37°C. The resulting thymocytes are typically >95% CD3+.

To identify new members of the PTPase gene family that are expressed in thymocytes, a PCT-based approach was used with degenerate oligonucleotides directed at conserved regions of the PTPase catalytic domain. A fragment of ~400bp was amplified from thymocyte cDNA and identified PCR amplified clones corresponding to seven different phosphatases. Six clones were identical to

previously isolated human phosphatases: PTP-PEST,²⁴ PTP1B,²⁵ TCPTP,²⁶ HPTPδ,⁶ CD45 and PTPMEG2.²⁷ A seventh clone had no human homologue but was 90% homologous to the murine phosphatase Z70PEP.¹⁰ This clone was used to screen a human thymocyte cDNA library. The first screening isolated two overlapping clones, P1 and P2 (Fig.1). Clone P2 was used to isolate a further three overlapping clones P3-P5 from the cDNA library. Assembly of the five overlapping clones revealed a single cDNA of 2300bp containing an open reading frame (ORF) of 2076bp, predicting a protein of 692 amino acids. The sequence surrounding the putative ATG/methionine start codon contained a purine (A) at position -3 and G at +4, both regarded as important criteria for an eucaryotic initiation site.³² The N-terminal region of the amino acid sequence (Fig 2) contained a single PTPase catalytic domain characterized by the conserved sequence motif (I/V)HCXXGXXRS/T. This sequence, thought to form the phosphate binding pocket for substrate, is found in all PTPases and is essential for their enzymatic activity. In addition to the 5 overlapping clones a single kb clone was isolated (P6, Fig. 1), with 200bp of its 5'-end overlapping nucleotides 1950-2055 of the complete cDNA previously isolated. However this was followed by an alternative 700bp, coding for an ORF totalling 2424bp. The long (3056bp) and short (2356bp) forms share nucleotides 1-2097 but code for alternative C-terminal sequence. These forms are designated Lyp1 and Lyp2 respectively. Lyp2 is an alternative spliced isoform of Lyp1.

Polymerase chain reaction and subcloning of the Phosphatase clones.

Total RNA was prepared from thymocytes using Trizol reagent (Gibco-BRL). First strand cDNA synthesis was performed with oligo-dt primer and Superscript II RT (Gibco-BRL). This was used as a template for PCR amplification with Taq DNA polymerase (Perkin Elmer Cetus) and the following degenerate primers:

PTP1: GCGGATCCTCIGA(C/T)TA(C/T)AT(A/C/T)AA(T/C)GC [sense, SEQ. ID NO: 5]

PTP2: GCGAATTCCCIACICGIGC(A/G)CT(G/A)CA(G/A)TG [antisense, SEQ. ID NO: 6].

These degenerate primers are designed to match two highly conserved sequences within PTPase catalytic domains, XDYINA and HCSAG/VG respectively. PCR was

performed as follows: five cycles of 60sec. at 94 °C, 30 sec. at 37 °C and 60 sec. at 72 °C , and a further 25 cycles with an annealing temperature of 45 °C. Fragments of approximately 400bp were isolated, cloned and sequenced.

Isolation and Sequencing of Lyp1 and Lyp2 cDNA clones.

An oligo-dT derived λ gt10 cDNA library from human thymocytes was screened with a [32 P] labelled 430bp Lyp1 fragment obtained by PCR. λ Plaques were transferred to ICN Biotrans nylon filters and screened by hybridization at 65 °C in 5 x SSC, 5 x Denhart's solution, 0.1%SDS (22). Phage DNA was prepared from positive plaques, cDNA inserts were excised, subcloned into pUC-19, and sequenced. To obtain the complete Lyp1 cDNA, secondary and tertiary library screenings were performed with the 1.3kb and 0.6kb partial cDNA clones isolated in the first screening (Fig. 1) . One clone (P5) from the second screening was found to contain the carboxyterminal sequence of the spliced form of Lyp1 (Lyp2).

Example 2 - Lyp2 production by alternative RNA splicing of the Lyp1 message.

To confirm the hypothesis that Lyp2 was produced by alternative splicing of Lyp1 RNA, three oligonucleotides matching sequences around the putative splicing sites were used in PCR amplifications on a genomic DNA template (Fig.4). Oligonucleotide 1 corresponded to the common nucleotides 2076-2097 of Lyp1 and Lyp2 (Table 1 and 2), oligonucleotide 2 to Lyp2 untranslated area adjacent to the stop codon (nucleotides 2150-2168), and oligonucleotide 3 to Lyp1 sequence immediately downstream of primer 1 (nucleotides 2098-2120) (Fig 4A). The resultant PCR products are shown in Fig 4B. PCR amplification with primers 1 and 3 created an approximately 3.5kb DNA fragment, suggesting the presence of an intron between the primers. However PCR with primers 1 and 2 resulted in a much smaller fragment of 100bp, the size expected from Lyp2 cDNA sequence. Upon sequencing, the 5' end sequence of the 3.5kb fragment was found to contain the alternative C-terminus, stop codon and untranslated nucleotide sequence of Lyp2 (Fig 4C). This clearly demonstrated that Lyp1 and Lyp2 are the alternatively spliced transcripts of a single gene. While the 3.5kb intron is spliced out of the Lyp1 form, this does not occur in the Lyp2 isoform and as a result only 7 amino acids are added and an alternative stop codon is utilized.

Example 3 - Characterization of Lyp1 and Lyp2 proteins

To determine whether Lyp1 and Lyp2 proteins are expressed at their predicted sizes or undergo processing in eukaryotic cells, the full length cDNAs were tagged at their 5' end with a haemagglutinin (HA) epitope and transfected into COS-7 cells. The cDNAs of Lyp1 and Lyp2 code for polypeptides of molecular weight (Mw) 92,000 and 78,000 respectively. On SDS-PAGE gel the molecular weights of the transfected proteins were close to the predicted values (Fig.7). Antibodies to the HA tag recognized a single protein with an apparent Mw of 96kDa in Lyp1 transfected cells and a single protein with an apparent molecular Mw of 80kDa in Lyp2 transfectants, indicating that these phosphatases do not undergo significant post translation modifications.

Determination of Actual Size of Lyp1 and Lyp2 Proteins

To determine the actual size of the Lyp1 and Lyp2 proteins, the full length cDNAs were cloned by PCR from oligo-dT selected mRNA, tagged with a T7 epitope and transfected into COS-7 cells. The deduced amino acid sequences of Lyp1 and Lyp2 predict molecular weights of 92 kD and 78 kD respectively. Immunoprecipitation of the transfected proteins with anti-T7 or anti-Lyp antibodies and blotting with the T7 antibody showed the protein Lyp2 to have an apparent molecular weight of 85 kD, slightly higher than the predicted molecular weight. Two proteins with apparent molecular weights of 96 kD and 105 kD were observed in COS-7 cells transfected with the Lyp1 cDNA (Fig. 6). Both of these proteins were recognized by the T7 and Lyp antibodies. The lower molecular weight product probably represents the result of proteolytic degradation while the 105 kD protein is intact Lyp1. When immunoprecipitated from lymphoid cells lines the native Lyp1 protein has an apparent molecular weight of 105 kD, in agreement with the size observed in transfected COS-7 cells (Fig. 7).

Preferential Lymphoid Expression of Lyp1 Transcripts

Cell preparation and cell lines

Lymphocytes were isolated from tonsil tissue or from peripheral blood of healthy volunteers by Ficoll-Hypaque centrifugation, following by rosetting with

neuraminidase treated sheep red blood cells (RBC) to isolate T lymphocytes. After isolating rosettes by FicollHypaque gradient centrifugation, T cells were released with ACT treatment (0.75% NH_4Cl in 20 mmol/L Tris, pH 7.2) of the rosettes to lyse the red blood cells. The buffy layer, containing the B cells, was washed three times with PBS. The resultant T lymphocytes are typically 98% to 99% CD3+ and the B lymphocytes are typically 97% to 98.5 % CD 19+.

To induce activation and maturation of peripheral T lymphocytes, 25×10^6 T cells were stimulated with 2.5 $\mu\text{g}/\text{ml}$ of anti-CD3 (Calbiochem) or 10 $\mu\text{g}/\text{ml}$ of phytohemagglutinin (PHA) (Gibco BRL) for 24 to 48 hours at 37°C in RPMI (10% FCS).

Northern blot analysis of mRNA from various human tissues using a Lyp cDNA probe common to both Lyp isoforms revealed a major transcript of approximately 4.4 kb in all of the lymphoid tissues examined (Fig.5). Substantial levels of Lyp mRNA were detected in spleen, thymus, lymph node, peripheral leukocytes, tonsil B and T lymphocytes, and to a lesser degree in bone marrow. In contrast, Lyp transcripts were not detected in prostate, ovary, testis fetal liver or colon tissues (or other human tissues including heart, lung, brain, placenta, or liver, data not shown). A low level of Lyp expression could however be detected in the small intestine and appendix mucosa, presumably due to the presence of contaminating lymphocytes. Lyp2 expression could not be detected by Northern blot analysis using a probe to the last 21 specific nucleotides. Therefore its expression, relative to Lyp1, was quantified by competitive PCR on polyA+-derived single strand thymocyte cDNA. The internal standards were constructed by deleting 140bp from both Lyp1 and Lyp2 cDNAs. Co-amplification of the target cDNA with various concentrations of internal control revealed that a concentration of 0.05×10^{-4} pM control DNA was needed to produce equivalent amounts of Lyp2 target and control PCR product, while 5×10^{-4} PM of internal standard was required with specific primers to Lyp1 (Fig.8). These results suggest that the level of Lyp1 expression in thymocytes is approximately 100 fold greater than the level of Lyp2 expression.

From its expression pattern in normal human tissues and cells, Lyp appears to be a predominantly lymphoid phosphatase, although a low level of expression could also be detected in the monocyte cell line. U937. Myeloid (OCI/AML3: origin and

properties previously described (55)) and erythroleukemia (K562) cell lines displayed little to no expression.

Northern blot analysis of Lyp1 and Lyp2 RNA.

To further characterize the expression of the Lyp isoforms, Northern blots were performed with a Lyp2 specific cDNA probe on human mRNA from lymphoid and hematopoietic tissues. This revealed a single 5.2 kb transcript in all of the tissues examined, with the highest level of expression in fetal liver (Fig. 5). Subsequent blotting of the same membrane with a Lyp1 specific probe revealed the dominant 4.4 kb transcript previously observed. Lyp1 demonstrated a high level of expression not only in the mature lymphoid tissues, but also in the thymus. In contrast to Lyp2, Lyp1 mRNA could not be detected in fetal liver and only a low level of expression could be seen in bone marrow.

For the actual northern blotting procedure, total RNA was extracted from thymocytes using Trizol reagent (Gibco BRL). Poly A+ RNA was isolated by two passages through an oligo(dt) column. 2µg of Poly A+ RNA per sample was electrophoresed in a 1% agarose formaldehyde gel and capillary blotted onto nitrocellulose filters. Filters and a human multiple tissue poly A+ RNA northern blots (Clontech) were hybridized overnight at 42 °C with [³²p] labelled Lyp cDNA probes in 50% formamide, 5 x SSC, 5 x Denhart's solution, 0.1% SDS, 50µl Na₂HP0₄ pH 6.5, and denatured Salmon sperm DNA (100µg/ml). Specifically, 2µg of poly A+ RNA from various human tissues was hybridized with a 1.3 kb cDNA probe common to both Lyp1 and Lyp2 and exposed 7 days or 24 hr with Actin. After hybridization, the final wash was performed in 0.2%SSC, 0.1%SDS at 55 °C (22).

Relative Quantification of Lyp1 and Lyp2 mRNA by Competitive Polymerase Chain Reaction.

The relative levels of Lyp1 and Lyp2 messenger RNA (mRNA) in thymocytes were quantified by competitive PCR using a synthetic cDNA as internal standard. This technique involves co-amplification of a target cDNA (produced from the corresponding mRNA by reverse transcription) and of the internal standard. The target cDNA and the internal standard use the same primer sequence, but yield PCR products of different

sizes that can be resolved on gel electrophoresis. In the exponential phase of the amplification, the amount of target cDNA can be quantified by comparison with the amplification of various amounts of the internal standard. The amount of target sequence in the sample is estimated by the amount of control producing an equivalent amounts of PCR products. The internal standards were constructed by deleting 140bp from both Lyp1 and Lyp2 cDNAs, using two EcoRI sites found in position 1805 and 1945. PCR primers: The 5' primer for both Lyp1 and Lyp2- corresponds to nucleotides 1660-1682 with the 3' primer for Lyp1- corresponding to nucleotides 2425-2447, while the 3' primer for Lyp2- corresponds to nucleotides 2075-2097. cDNA was prepared from oligo (dT) selected mRNA as described previously. Aliquots of thymus cDNA were co amplified with varying amounts of internal standard for 26 cycles for Lyp1 and 35 cycles for Lyp2. (denaturing 94 °C 30 sec., annealing at 54 °C and elongation 45 sec. at 72 °C). The PCR products (40µl) were electrophoresed on 1.2% agarose gel, stained with ethidium bromide and photographed. The possibility of genomic DNA contamination in the RT PCR reaction was excluded with the appropriate controls.

Example 4 - Cellular localization of Lyp1 and Lyp2 in Transfected COS-7 cells

In order to determine the cellular localization of the two phosphatases, the distribution of both Lyp1 and Lyp2 was determined by indirect immunofluorescence in transiently transfected COS-7 cells. Lyp1 and Lyp2 were inserted into the pcDNA3 eucaryotic expression vector (Invitrogen) and a T7 tag or HA epitope (YPYDVPDYA), as a three-tandem repeat, inserted at the 5' end of the coding sequences of both Lyp1 and Lyp2 cDNAs. Constructs were verified by sequencing. COS-7 cells were transfected with 2µg DNA and 17µl of Lipofectamine for 5 hours, incubated on sterile cover slips in six well plates (0.3×10^6 /plate) in DMEM containing 10% fetal calf serum and stained 48 hours post transfection. The COS-7 cells were then washed in PBS and fixed for 30 min at room temperature in 2% paraformaldehyde. Cell permeabilization was performed with 0.1% Triton X100 and after blocking non-specific sites with 5% donkey serum, the cells were incubated with monoclonal anti-HA (1:1000) from Boehringer-Mannheim, for 60 min at room temperature. The cells were washed and exposed for 45 min to cy3 conjugated affinipure Donkey anti-mouse IgG (1:1000 in PBS) from Jackson ImmunoResearch Laboratories Inc. After 3 to 4 washes, immunoreactivity was detected

by fluorescence microscopy. COS-7 cells transfected with either Lyp1 and Lyp2 displayed prominent perinuclear and cytoplasmic staining but no staining of the nucleus (Fig. 9). No fluorescence was noted in COS-7 cells transfected with vector alone. The pattern of staining suggests that both of these phosphatases are predominantly cytoplasmic.

Transfection

To examine the actual size of the expressed proteins Lyp1 and Lyp2, cDNAs were inserted into the pCDNA3 eukaryotic expression vector (Invitrogen). An HA epitope (YPYDVPDYA) derived from the haemagglutinin protein of influenza virus, was inserted as three-tandem repeat at the 5' end of the coding sequences of both Lyp forms. The constructs were verified by sequencing. COS-7 cells (0.5×10^6) were transfected with 5µg plasmid DNA in 50µl of Lipofectamine (Gibco -BRL) for 5 hours according to the manufacture's instructions. 24 hours before transfection 0.5×10^6 COS-7 cells were plated on 60mm plates in Dulbaco's modified Eagle medium (DMEM) containing 10% fetal calf serum. To examine the cellular localization of the expressed proteins, Cos-7 cells were transfected with 2µg DNA and 17µl of Lipofectamine for 5 hours, incubated on sterile cover slips in six well plates (0.3×10^6 /plate) in DMEM containing 10% fetal calf serum for 48 hours and stained. 48 hours post transfection the COS-7 cells were harvested and solubilized in cold lysis buffer (20mM Tris pH 7.5, 150 mM NaCl, 1mM EDTA, 1% NP 40 and 1mM PMSF).

Immunoprecipitation and Western Blotting

For NP-40 cells, 1% NP-40 cell lysates were pre-cleared by centrifugation. Immunoprecipitation of T7 tagged Lyp was carried out by the addition of 1µg of T7 antibody, or by the addition of 5µl of the Lyp anti-serum followed by the addition of 20µl of a 50:50 suspension of protein G sepharose (Pharmacia) and incubation overnight at -4°C. Immunoprecipitates were washed three times with lysis buffer and separated by 6% SDS-PAGE. The separated proteins were electrophoretically transferred to Hybond C Super nitro-cellulose membrane (Amersham Life Science). Membranes were blocked with 5% non-fat milk and blotted with anti-T7 (1:10,000) or with anti Lyp (1:800). Detection was performed with horseradish peroxidase conjugated second antibodies

from Amersham Life Science and chemiluminescence reagent from Kirkeggard & Perry Laboratories.

For COS-7 cells, the cells (0.5×10^6 /plate) were washed three times with cold PBS and solubilized in cold lysis buffer (20mM Tris pH 7.5, 150mM NaCl, 1mM EDTA, 1% NP-40, 1mM PMSF). The lysates were cleared by centrifugation. SDS sample buffer was added to the clarified lysates and resolved by 7% SDS-PAGE. The proteins were electrophoretically transferred to Hybond-C super nitrocellulose membrane (Amersham Life Science). Membranes were blocked with 5% non fat milk and probed with HA monoclonal antibody from Blco-Berkely. Detection was performed with horseradish peroxidase conjugated sheep anti mouse purchased from (Amersham Life Science) and chemiluminescence reagent from Kirkeggard & Perry laboratories.

Indirect Immunofluorescence

48 hours post transfection Cos-7 cells were washed in PBS and fixed for 30 min at room temperature in 2% paraformaldehyde. Cell permeabilization was performed with 0.1% Triton X100. After blocking non-specific sites with 5% Donkey serum, the cells were incubated for 60 min at room temperature with monoclonal anti HA tag (1:1000 in PBS) from Blco-Berkely. The cells were washed and exposed for 45 min. to cy3 conjugated affinipure Donkey anti mouse IgG (1:1000 in PBS) from Jackson Immunoresearch Laboratories, Inc. After 3-4 washes immunoreactivity was detected by fluorescence microscopy.

Example 5 - Characterization of Lyp Protein Expression

Cell Lines

The G2 pre-preB cell line was derived from a patient with acute lymphocytic leukemia (56). All of the other cell lines used for the present invention were obtained from the American Type Culture Collection. All cells were maintained in RPMI 1640 containing 10% fetal calf serum.

Antibodies

Rabbit polyclonal antibodies were raised to a mixture of two peptides of Lyp, with the amino acid sequences RTKSTPFELIQQR and SKMSLDLPEKQDG. These peptides were chosen from a potentially exposed area, as predicted by Hopp and Woods, in the non-catalytic domain. A second polyclonal antibody was raised to a bacterial fusion protein of the catalytic domain of Lyp (Pet vector - Novagen). After careful testing these antibodies were used for immunoprecipitation and western blotting. T7 antibody was purchased from Novagen (WI), anti-cbl, anti-Jak3 and anti-p110 from Santa Cruz Biotech (CA) and anti-phosphotyrosine from U.B.I. (NY).

The above-described polyclonal antibodies were first used to characterize the expression of Lyp proteins in human hematopoietic cell lines (Fig. 10). A single band of 105 kD is seen in both T cell (Jurkat) and B cell lines (Daudi and Ramos), the same size as observed upon transfection of Lyp1 cDNA into COS-7 cells (Fig. 6). Lyp1 expression could not be detected in either the monocytic (U937) or myeloid (K562) cell lines, while low levels of expression could be seen in pre-B cells (G2, A1). This pattern of protein expression correlates with that of Lyp1 mRNA observed by Northern blotting. A protein of the predicted size of Lyp2 (85 kD) in the cell lines examined was not detected.

Expression of the Lyp protein in primary lymphoid cells (Fig. 11) was also examined. Both thymocytes and tonsil T lymphocytes expressed Lyp1, while resting T cells from peripheral blood, in addition to expressing low levels of Lyp1, also expressed an 85 kD protein, recognized by both polyclonal Lyp antibodies. This is the predicted molecular weight of Lyp2, the shorter alternatively spliced form of Lyp1.

To determine whether expression of the Lyp proteins may be regulated by activation in T cells, normal peripheral blood T lymphocytes were incubated with either PHA, or anti-CD3 and harvested after 24 or 48 hours (Fig. 11B). An increase in the level of Lyp1 protein expression was observed after 24 hours of either stimulus, with a further increase seen after 48 hours with anti-CD3. The 85 kD protein could no longer be detected after a 24 hours incubation with either PHA or anti-CD3.

Example 6 - Identifying the Chromosomal Location of Lyp

A 1.8 kb Lyp cDNA fragment was used as a probe to examine the chromosomal location of Lyp using fluorescent *in situ* hybridization. The regional assessment of this

cDNA probe was determined by the analysis of 40 well-spread metaphases. Biotinylated Lyp probe was prepared by nick translation for fluorescence *in situ* hybridization (FISH) to normal human lymphocyte chromosomes (counterstained with propidium iodide and 4',6-diamidin-3-phenylindol-dihydrochloride, DAPI, according to published methods (43, 44). The probe was detected with avidin-fluorescein isothiocyanate (FITC) followed by biotinylated anti-avidin antibody and avidin-FITC. Images of metaphase preparations were captured by thermoelectrically cooled charge coupled camera (Photometrics, Tucson, AZ). Separate images of DAPI banded chromosomes (45) and FITC targeted chromosomes were obtained and merged electronically using image analysis software (Yale University, New Haven, CT) and pseudo coloured blue (DAPI) and yellow (FITC) as described by Boyle et al., (44). The band assignment was determined by measuring the fractional chromosome length and by analyzing the banding pattern generated by the DAPI counterstained image (46).

Positive hybridization signals at the short arm of human chromosome 1 in region p13 (shown schematically in Fig. 3) were noted in approximately 10% of the cells. The band assignment was determined by measuring the fractional chromosomal length and by analyzing the banding pattern generated by DAPI counterstained image. The low frequency of hybridization obtained with this probe is commonly seen with small cDNA probes of this size. Signals were visualized on both homologues in 90% of the positive spreads (Fig. 3). No fluorescence signal was seen on any other chromosome, implying that the human Lyp gene is located on chromosome 1 in the p13 region.

Example 7 - Phosphatase Assay

To determine whether Lyp1 possessed a catalytically active tyrosine phosphatase domain, COS cells were transfected with T7-LyP cDNA, the protein immunoprecipitated with anti-T7 and used to dephosphorylate a labelled synthetic peptide, Raytide, in an *in vitro* phosphatase assay. Raytide peptide was ³³P labelled on tyrosine residues *in vitro* using the tyrosine kinase p60src and purified on phosphocellulose paper. Release of ³³P over time was measured in the phosphatase assay and compared to controls from untransfected cells. The results showed a seven fold increase in ³³P release from the substrate incubated with Lyp immunoprecipitates compared to control immunoprecipitates (Fig. 12), demonstrating that Lyp does possess

tyrosine phosphatase activity. This activity can be completely inhibited by pervanadate (data not shown).

Specifically, the synthetic peptide Raytide was phosphorylated according to the method described by Guan et al., (1994)(54), on tyrosine by p60src (Oncogene Science) as follows: 10 µg Raytide in 50mM Hepes pH 7.5, 10 mM MgCl₂, 0.067% β-mercaptoethanol, 0.05 mM ATP was incubated with 300 µCi ³³P ATP per ml and 2µg p60src in a final volume of 30µl. The reaction was allowed to proceed for 30 minutes at 30°C and was stopped by the addition of 120µl 10% phosphoric acid.

The sample was spotted onto two 1 x 1cm sheets of P81 phosphocellulose paper and extensively washed with 0.5% phosphoric acid. Phosphorylated peptide was eluted twice with 1 ml 500mM (NH₄)₂CO₃, lyophilized and resuspended in 100µl H₂O.

The phosphorylated substrate was used in the phosphatase assay as described by Stueli et al (1989)(54). The phosphatase assay mixture, 50 µl, contains 5 µl of x10 phosphatase (250mM Hepes pH 7.3, 50mM EDTA, 100mM dithiothreitol), 5µl of radioactive substrate (Raytide) and 5µl sample (Lyp immunoprecipitate) and H₂O to final volume. The assay was allowed to proceed at 30°C for the indicated time and the reaction terminated by the addition of 750µl of a charcoal mixture (0.9M HCl, 90mM sodium pyrophosphate, 2mM NaH₂PO₄, 4% v/v Norit A). After centrifugation the free ³³P in the supernatant was measured.

Example 8 - Determination of Involvement of Lyp1 in TCR Signalling

One of the earliest events following TCR stimulation of T cells is the induction of tyrosine phosphorylation. In order to determine whether Lyp played a role in TCR signalling, human thymocytes were stimulated with anti-CD3 for various periods of time, Lyp immunoprecipitated and blotted with anti-phosphotyrosine. This revealed that while Lyp itself is not detectably tyrosine phosphorylated, a heavily phosphorylated protein of 116-120 kD co-precipitates with Lyp, appearing within 1 minute of stimulation (Figure 13A). Once activated, the phosphorylation level of this protein remained constant over a period of 20 minutes. The 116 kD phosphorylated protein was identified by western blotting of Lyp immunoprecipitates from CD3 stimulated thymocytes with antibodies to various candidate proteins. The 116 kD protein associated with Lyp1 was found to be c-Cbl (Fig. 13B), but not p125Fak, p116 Jak3 or

p110 PI3-kinase. No alteration in the amount of Cbl co-immunoprecipitating with Lyp could be detected upon anti-CD3 stimulation, suggesting that Lyp1 and Cbl are constitutively associated, although Cbl can be inducibly phosphorylated. This interaction was also observed in the mature T cell line Jurkat (not shown) and further confirmed by transfection of Lyp1 into COS-7 cells and examining its association with the endogenous Cbl protein (Fig. 13C). Lyp1 was found not only to co-precipitate with Cbl in COS cells, but also to reduce significantly the basal level of Cbl tyrosine phosphorylation (Fig. 13D). This suggests that Lyp1 may serve to regulate Cbl function and possibly that of Cbl associated proteins in lymphoid cells.

Example 9

cDNA for the phosphatase Lyp1 and the indicated kinase, in the eucaryotic expression vector pcDNA3, were transiently transfected into either the COS-7 monkey epithelial cell line (A) or the 293T human epithelial cell line as indicated. 48 hours after transfection, cells were harvested, lysates made in 1% NP-40 lysis buffer and immunoprecipitations performed with antibodies to the transfected kinase. Immunoprecipitates were washed, boiled in SDS sample buffer and electrophoresed on SDS-PAGE. After electro-transfer to nitrocellulose membrane, Western blotting was performed with anti-phosphotyrosine antibodies and chemiluminescent detection reagents. In both COS-7 and 293-T cells, Lyp1 co-transfection clearly resulted in a reduction in Zap-70 phosphorylation while Fyn was unaffected. Lyp1 could also down-regulate Zap-70 after its activation by Fyn in 293-T cells (B, lanes 3 and 4). The closely related Zap family kinase Syk was also unaffected by Lyp1 (C). The results are shown in Figure 14.

Example 10

cDNA for the phosphatase Lyp1 and the indicated kinase, in the eucaryotic expression vector pcDNA3, were transiently transfected into the COS-7 monkey epithelial cell line. 48 hours after transfection, cells were harvested, lysates made in 1% NP-40 lysis buffer and immunoprecipitations performed with antibodies to the transfected kinase. Immunoprecipitates were washed, boiled in SDS sample buffer and electrophoresed on SDS-PAGE. After electro-transfer to nitrocellulose membrane,

Western blotting was performed with anti-phosphotyrosine antibodies and chemiluminescent detection reagents.

Lyp1 clearly reduced the tyrosine phosphorylation of Jak3 (C), while having little effect upon Syk (D), possibly increasing its phosphorylation slightly; an effect not seen when Syk is co-transfected with a catalytically inactive form of Lyp1 (Lyp-N, where Cysteine 227 is replaced by Serine).

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TABLE 1

TCCCTCAACCTACTTATAGACTATTTTTCTTGCTCTGCAGCATGGACCAAAGAGAAATTCT
 GCAGAAGTTCCTGGATGAGGCCCAAAGCAAGAAAATTACTAAAGAGGAGTTTGCCAATG
 AATTTCTGAAGCTGAAAAGGCAATCTACCAAGTACAAGGCAGACAAAACCTATCCTACAA
 CTGTGGCTGAGAATGCCAAGAATATCAAGAAAAACAGATATAAGGATATTTGCCCTATG
 ATTATAGCCGGGTAGAACTATCCCTGATAACCTCTGATGAGGATTCCAGCTACATCAATG
 CCAACTTCATTAAGGGAGTTTATGGACCCAAGGCTTATATTGCCACCCAGGGTCCTTTATC
 TACAACCCCTCCTGGACTTCTGGAGGATGATTTGGGAATATAGTGTCTTATCATTGTTATG
 GCATGCATGGAGTATGAAATGGGAAAGAAAAGTGTGAGCGCTACTGGGCTGAGCCAGG
 AGAGATGCAGCTGGAATTTGGCCCTTTCTCTGTATCCTGTGAAGCTGAAAAAAGGAAATC
 TGATTATATAATCAGGACTCTAAAAGTTAAGTTCAATAGTGAAACTCGAACTATCTACCA
 GTTTCATTACAAGAATTGGCCAGACCATGATGTACCTTCACTATAGACCCTATTCTTGAG
 CTCATCTGGGATGTACGTTGTTACCAAGAGGATGACAGTGTTCCCATATGCATTCACTGCA
 GTGCTGGCTGTGGAAGGACTGGTGTATTTGTGCTATTGTTGATTATACATGGATGTTGCT
 AAAAGATGGGATAATTCCTGAGAACTTCAGTGTTTTTCAGTTTGATCCGGGAAATGCGGAC
 ACAGAGGCCTTCATTAGTTCAAACGCAGGAACAATATGAACTGGTCTACAATGCTGTATT
 AGAACTATTTAAGAGACAGATGGATGTTATCAGAGATAAACATTCTGGAACAGAGAGTCA
 AGCAAAGCATTGTATTCCTGAGAAAAATCACACTCTCCAAGCAGACTCTTATTCTCCTAAT
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 AGTTTTGCACCCTGCTAAATCAAGCACTTCTTTTGACTTTCTGGAGCTAAATTACAGTTTT
 GACAAAAATGCTGACACAACCATGAAATGGCAGACAAAGGCATTTCCAATAGTTGGGGA
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 AAATCAACTCCTTTTGAATTGATACAGCAGAGAGAAACCAAGGAGGTGGACAGCAAGGA
 AAACCTTTCTTATTTGGAATCTCAACCACATGATTCTTGTGTTTGTAGAGATGCAGGCTCAA
 AAAGTAATGCATGTTTCTTCAGCAGAACTGAATTATTCAGTCCCATATGACTCTAAACACC
 AAATACGTAATGCCTCTAATGTAAAGCACCATGACTCTAGTGCTCTTGGTGTATATTCTTA
 CATACCTTAGTGGAATAATCCTTATTTTTCATCATGGCCTCCAAGTGGTACCAGTTCTAAG
 ATGTCTCTTGATTTACCTGAGAAGCAAGATGGAAGTGTCTTCTCTCTGTTGCCAA
 CATCCTCTACATCCCTCTTCTTATTACAATTCACATAGTTCTTTATCACTGAATTCTCCA
 ACCAATATTTCTCACTATTGAACCAGGAGTCAGCTGTACTAGCAACTGCTCCAAGGATA
 GATGATGAAATCCCCCTCCACTTCCTGTACGGACACCTGAATCATTTATTGTGGTTGAGG
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 TACTTAGACCAAGCAAGAGTGTAAAACTCCGAAGTCCTAAATCAGAACTACATCAAGATC
 GTTCTTCTCCCCACCTCCTCTCCCAGAAAGAACTCTAGAGTCCTTCTTTCTTGCCGATGA
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 CGTTTTTCAAAACCCAAAGGACCAAGGAATCCACCACCAACTTGAATATTTAATAAAAC
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 TTGAAAACCTGCAATAAAAGTTTGTCACTTGAGCTTATGTACAGAATGCTATATGAGAAA
 CACTTTTAGAATGGATTTATTTTTCATTTTGGCAGTTATTTTATTTTCTTTTACTTTTCTA
 CATAAACATAAACTTCAAAAGGTTTGTAAAGATTGGATCTCACTAATTTCTACATTGCCA
 GAATATACTATAAAAAGTTAAAAAATAAACTTACTTTGTGGGTTGCAATACAACTGCT
 CTTGACAATGACTATTCCCTGACAGTTATTTTGCCTAAATGGAGTATACCTTGTAATCT
 TCCCAAATGTTGTGGAATACTGGAATATTAAGAAAATGAGAAATTATTTTATTAGAATA
 AAATGTGCAATAATGACAATTATTTGAATGTAACAAG

TABLE 2

MDQREILQKFLDEAQSKKITKEEFANEFLKLRQSTKYKADKTYPTTVAENAKNIKKNRYKDI
 LPYDYSRVELSLITSDDEDSSYINANFIKGVYGPAYIATQGPLSTLLDFWRMIWEYSVLIIVMA
 CMEYEMGKKKCERYWAEPGEMQLEFGPFSVSCEAEKRKSDYIIRTLKVKFNSETRTIYQFHYK
 NWPDHDVPSSIDPILELIWDVRCYQEDDSVPICIHCSAGCGRTGVICAIVDYTWMLLKDGIIPEN
 FSVFSLIREMRTQRPSLVQTQEYELVYNAVLELFKRQMDVIRDKHSGTESQAKHCPEKNHTL
 QADSYSPNLPKSTTKAAKMMNQQRITKMEKESSSDFRTSEISAKEELVLHPAKSSTSDFLEL
 NYSFDKNADTTMKWQTKAFPIVGEPLQKHQSLDLGSLLFEGCSNSKPVNAAGRYFNSKVPITR
 TKSTPFELIQQRETKEVDSKENFSYLESQPHDSCFVEMQAQKVMHVSSAELNYSLPYDSKHQIR
 NASNVKHHDSALGVYSYIPLVENPYFSSWPPSGTSSKMSLDLPEKQDGTVPSSLLPTSSTSLF
 SYYNSSHSLSLNSPTNISSLLNQESAVLATAPRIDDEIPPLPVRTPEFIVVEEAGEFSPNVPKSL
 SAVKVKIGTSLEWGGTSEPCKFDDSVILRPSKSVKLRSKSELHQDRSSPPPLPERTLESFFLAD
 EDCMQAQSIETYSTSYPD TMENSTSSKQTLKTPGKSFTRSKSLKILRNMKKSICNSCPPNKPAES
 VQSNSSSFLNFGFANRFSKPKGPRNPPPTWNI

TABLE 3

TCCCTCAACCTACTTATAGACTATTTTTCTTGCTCTGCAGCATGGACCAAAGAGAAATTCT
 GCAGAAGTTCCTGGATGAGGCCCAAAGCAAGAAAATTACTAAAGAGGAGTTTGCCAATG
 AATTTCTGAAGCTGAAAAGGCAATCTACCAAGTACAAGGCAGACAAAACCTATCCTACAA
 CTGTGGCTGAGAATGCCAAGAATATCAAGAAAAACAGATATAAGGATATTTTGCCCTATG
 ATTATAGCCGGGTAGAACTATCCCTGATAACCTCTGATGAGGATTCCAGCTACATCAATG
 CCAACTTCATTAAGGGAGTTTATGGACCCAAGGCTTATATTGCCACCCAGGGTCCTTTATC
 TACAACCCTCCTGGACTTCTGGAGGATGATTTGGGAATATAGTGTCTTATCATTGTTATG
 GCATGCATGGAGTATGAAATGGGAAAGAAAAAGTGTGAGCGCTACTGGGCTGAGCCAGG
 AGAGATGCAGCTGGAATTTGGCCCTTTCTCTGTATCCTGTGAAGCTGAAAAAAGGAAATC
 TGATTATATAATCAGGACTCTAAAAGTTAAGTTCAATAGTGAAACTCGAACTATCTACCA
 GTTTCATTACAAGAATTGGCCAGACCATGATGTACCTTCATCTATAGACCCTATTCTTGAG
 CTCATCTGGGATGTACGTTGTTACCAAGAGGATGACAGTGTTCCTCATATGCATTCAGTGA
 GTGCTGGCTGTGGAAGGACTGGTGTATTTGTGCTATTGTTGATTATACATGGATGTTGCT
 AAAAGATGGGATAATTCCTGAGAACTTCAGTGTTCCTCAGTTTGATCCGGGAAATGCGGAC
 ACAGAGGCCCTTCATTAGTTCAAACGCAGGAACAATATGAACTGGTCTACAATGCTGTATT
 AGAACTATTTAAGAGACAGATGGATGTTATCAGAGATAAACATTCTGGAACAGAGAGTCA
 AGCAAAGCATTGTATTCCTGAGAAAAATCACACTCTCCAAGCAGACTCTTATTCTCCTAAT
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 AATCAAAGAATCTTCTCTCTTTGACTTTAGGACTTCTGAAATAAGTGCAAAAGAAGAGCT
 AGTTTTGCACCCTGCTAAATCAAGCACTTCTTTTGACTTCTGGAGCTAAATTACAGTTTT
 GACAAAAATGCTGACACAACCATGAAATGGCAGACAAAGGCATTTCCAATAGTTGGGGA
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 TCTAAACCTGTAAATGCAGCAGGAAGATATTTAATTCAAAGGTGCCAATAACACGGACC
 AAATCAACTCCTTTTGAATTGATACAGCAGAGAGAAACCAAGGAGGTGGACAGCAAGGA
 AAACCTTTCTTATTTGGAATCTCAACCACATGATTCTTGTTTTGTAGAGATGCAGGCTCAA
 AAAGTAATGCATGTTTCTTCAGCAGAAGTGAATTATTCAGTCCCATATGACTCTAAACACC
 AAATACGTAATGCCTCTAATGTAAAGCACCATGACTCTAGTGCTCTTGGTGTATATTCTTA
 CATACTTTAGTGGAAAAATCCTTATTTTCATCATGGCCTCCAAGTGGTACCAGTTCTAAG
 ATGTCTCTTGATTTACCTGAGAAGCAAGATGGAAGTGTTCCTTCTCTCTGTTGCCAA
 CATCCTCTACATCCCTCTTCTCTTATTACAATTCACATAGTTCTTTATCACTGAATTCTCCA
 ACCAATATTTCTCTACTATTGAACCAGGAGTCAGCTGTACTAGCAACTGCTCCAAGGATA
 GATGATGAAATCCCCCTCCACTTCCTGTACGGACACCTGAATCATTTATTGTGGTTGAGG
 AAGCTGGAGAATTCTACCAAATGTTCCCAAATCCTTATCCTCAGCTGTGAAGGTAAAAA
 TTGGAACATCACTGGAATGGGGTGGAAACATCTGAACCAAAGAAATTTGATGACTCTGTGA
 TACTTAGACCAAGCAAGAGTGTAAAACTCCGAAGTCCTAAATCAGGTAAAAATTTCTCTT
 GGCTTTAGATGACATTTAGCCCTAAGATTGGAAGAATGGTTCGTTAAGTTTAGAGTAATT
 CACTTCAGGAAGTTACTTGGTTCCCATAAATAGCTTCAGTATTCATTGATTTATTTCTGGC
 TTTCCCAGACTAGAAATTTGTAAAGAGTCATGGGGGAAGCTAGGGCTAACAGAAAAATA
 AAATAAAAATAATGGGATAAAAAATCGGAAGTACTGTTTTCCCCCTAGTCGGAGCACATC
 CGG

MDQREILQKFLDEAQSKKITKEEFANEFLKLRQSTKYKADKTYPTTVAENAKNIKNRYKDI
LPYDYSRVELSLITSDEDSSYINANFIKGVYGPKEYIATQGPLSTLLDFWRMIWEYSVLIIVMA
CMEYEMGKKKCERYWAEPEGMLEFGPFSVSCEAEKRKSDYIIRTLKVKFNSETRTIYQFHYK
NWPDHDVPSSIDPILELIWDVRCYQEDDSPICIHCSAGCGRTGVICAIVDYTWMLLKDGIIPEN
FSVFSLIREMRTQRPSTVQTQEYELVYNAVLELFRQMDVIRDKHSGTESQAKHCIPEKNHTL
QADSYSPNLPKSTTKAAKMMNQRTKMEIKESSSDFRTSEISAKEELVLHPAKSSTSDFLEL
NYSFDKNADTTMKWKQTKAFPIVGEPLQKHQSLDLGSLLEFGCSNSKPVNAAGRYFNKVPITR
TKSTPFELIQRETKEVDSKENFYSLESPHDSFCFVEMQAQKVMHVSSAELNYSLPYDSKHQIR
NASNVKIHDDSSALGVYSYIPLVENPYFSSWPPSGTSSKMSLDLPEKQDGTVPFSSLLPTSSTSLF
SYYNSDSSLNSPTNISSLLNQESA VLATAPRIDDEIPPLPVRTPESFIVVEEAGEFSPNPVKSLS
SAVKVKIGTSLEWGGTSEPKKFDDSVILRPSKSVKLRSPKSGKNFSWL

We claim:

1. An isolated polynucleotide comprising a nucleotide sequence encoding a Lyp protein.
2. The polynucleotide of claim 1 wherein the polynucleotide is cDNA, DNA, genomic DNA, RNA or mRNA.
3. The polynucleotide of claim 1 wherein the nucleotide sequence encodes a human Lyp protein.
4. The polynucleotide of claim 3 wherein the nucleotide sequence is selected from the group consisting of
 - (a) a nucleotide sequence encoding the amino acid sequence of Table 2 (Sequence ID NO:2), or a splice variant thereof; and
 - (b) a nucleotide sequence encoding the amino acid sequence of Table 4 (Sequence ID NO:4).
5. The polynucleotide sequence of claim 4 wherein the nucleotide sequence is selected from the group consisting of
 - (a) the nucleotide sequence of Table 1 (Sequence ID NO:1);
 - (b) the nucleotide sequence of Table 3 (Sequence ID NO:3);
 - (c) a nucleotide sequence complementary to the nucleotide sequence of (a) or (b);
 - (d) a nucleotide sequence which is a degeneracy equivalent of the nucleotide sequence of (a) or (b); and
 - (e) a nucleotide sequence which hybridises under stringent conditions to a nucleotide sequence of (a) or (b).

6. An isolated polynucleotide which encodes a Lyp protein having an amino acid sequence of greater than 70% overall identity to the amino acid sequence of Table 2.
7. An isolated polynucleotide which encodes a Lyp protein having an amino acid sequence of greater than 70% overall identity to the amino acid sequence of Table 4.
8. An isolated polynucleotide which encodes a Lyp protein having an amino acid sequence with at least 80% overall identity, preferably at least 90% overall identity to the amino acid sequence of Table 2.
9. An isolated polynucleotide which encodes a Lyp protein having an amino acid sequence with at least 80% overall identity, preferably at least 90% overall identity to the amino acid sequence of Table 4.
10. A nucleotide sequence comprising at least 10, preferably 15 and more preferably 20 consecutive nucleotides of Sequence ID NO:1 or Sequence ID NO:3.
11. A recombinant vector comprising a polynucleotide of any of claims 1 to 10.
12. A host cell comprising the recombinant vector of claim 11.
13. A substantially purified Lyp protein.
14. The protein of claim 13 which is a human Lyp protein.
15. The protein of claim 13 wherein the protein comprises an amino acid sequence selected from
(a) the amino acid sequence of Table 2 (Sequence ID NO:2); and

(b) the amino acid sequence of Table 4 (Sequence ID NO:4).

16. A substantially purified protein having an amino acid sequence of greater than 70% overall identity to the amino acid sequence of Table 2.

17. A substantially purified protein having an amino acid sequence of greater than 70% overall identity to the amino acid sequence of Table 4.

18. A substantially purified protein having an amino acid sequence with at least 80% overall identity, preferably at least 90% overall identity, to the amino acid sequence of Table 2.

19. A substantially purified protein having an amino acid sequence with at least 80% overall identity, preferably at least 90% overall identity, to the amino acid sequence of Table 4.

20. A peptide comprising at least 5, preferably 10, more preferably 20 consecutive amino acids of Sequence ID NO:2 or Sequence ID NO:4.

21. A peptide comprising at least one functional domain of a Lyp protein.

22. A peptide comprising at least one antigenic determinant of a Lyp protein.

23. An antibody which binds specifically to a Lyp protein.

24. The antibody of claim 23 which is a monoclonal antibody.

25. The antibody of claim 23 which is a polyclonal antibody.
26. A hybridoma cell that produces the antibody of claim 16.
27. A method for producing a Lyp protein comprising culturing the host cell of claim 12 under conditions in which the Lyp protein is expressed and isolating the Lyp protein therefrom.
28. A method for screening a candidate compound for an ability to increase or decrease the phosphatase activity of a Lyp protein comprising
- (a) providing an assay system for assaying Lyp phosphatase activity;
 - (b) assaying Lyp phosphatase activity in the presence or absence of the candidate compound; and
 - (c) determining whether the Lyp phosphatase activity was higher or lower in the presence of the candidate compound than in its absence.
29. A method for screening a candidate compound for ability to modulate expression of a Lyp gene comprising
- contacting a cell with a candidate compound, wherein the cell includes a regulatory region of a Lyp gene operably joined to a coding region; and
 - detecting a change in expression of the coding region.
30. A non-human animal wherein a genome of said animal, or of an ancestor thereof, has been modified by a modification selected from the group consisting of:
- (a) knockout of a Lyp gene; and
 - (b) insertion of a polynucleotide encoding a heterologous Lyp gene.

31. The animal of claim 30 wherein the polynucleotide encodes the amino acid sequence of Table 2 (Sequence ID NO:2) or Table 4 (Sequence ID NO:4).

32. The animal of claim 31 wherein the polynucleotide comprises the nucleic acid sequence of Table 1 (Sequence ID NO:1) or Table 3 (Sequence ID NO:3).

33. A pharmaceutical composition comprising an active ingredient selected from the group consisting of:

- (a) an isolated nucleotide sequence encoding a Lyp protein;
- (b) a substantially purified Lyp protein;
- (c) a substantially purified antibody which binds specifically to a Lyp protein

and a pharmaceutically acceptable carrier.

34. A method for treating a subject having a deficiency of Lyp activity comprising administering to the subject an effective amount of an agent selected from the group consisting of:

- (a) an isolated nucleotide sequence encoding a Lyp protein;
- (b) a substantially purified Lyp protein.

35. A method for modulating signalling mediated by the T cell receptor, the method comprising administering to a T cell an agent which increases Lyp phosphatase activity or increases Lyp expression in the T cell.

36. A method for reducing or preventing T cell activation and/or proliferation, the method comprising administering to the T cell an agent which increases Lyp phosphatase activity or increases Lyp expression in the T cell.

37. A method for treating a disorder which requires

immunosuppression, the method comprising administering to the subject in need of treatment an immunosuppression-effective amount of an agent which increases Lyp phosphatase activity or increases Lyp expression.

38. A method for treating lymphoma in a subject, the method comprising administering to the subject an agent which increases Lyp phosphatase activity or increases Lyp expression in an amount effective to reduce or prevent lymphoma cell proliferation.

39. A method for preventing or treating a disorder characterised by an abnormality in the T cell receptor signalling pathway or the IL2-mediated signalling pathway, comprising modulating signalling by administration of an agent which increases Lyp phosphatase activity or Lyp expression.

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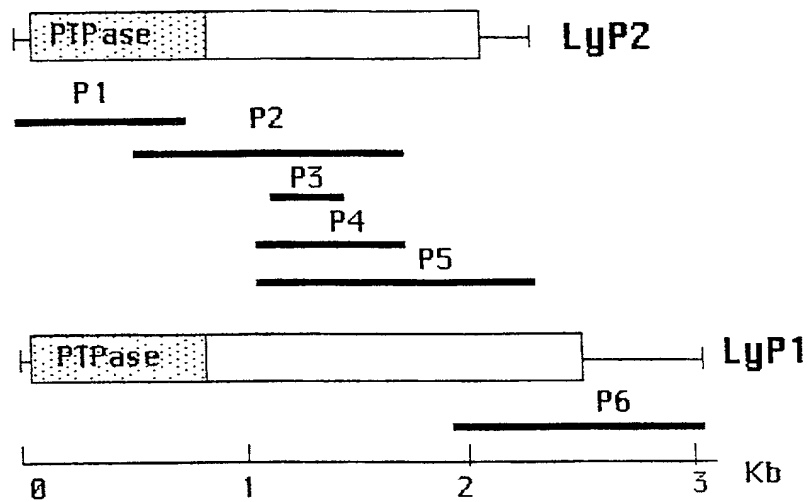


FIGURE 1

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LvP12	MDQREILQKF LDEAQS K KIT KEEFAN E FLK LKRQSTKYKA DKTYPTTVAE	50
PEP	MDQREILQQL LKEAQKKKLN SEEFAS E FLK LKRQSTKYKA DKTYPTTVAQ	50
Consensus	MDQREILQ.. L.EAQ.KK.. .EEFA.EFLK LKRQSTKYKA DK.YPTTVA.	50
LvP12	NAKNIKKNRY KDILPYDYSR VELSLITSDE DSSYINANFI KGVYGP K AYI	100
PEP	RPKNIKKNRY KDILPYD H SL VELSLITSDE DSSYINASFI KGVYGP K AYI	100
Consensus	..KNIKKNRY KDILPYD.S. VELSL.TSDE DSSYINA.FI KGVYGP K AYI	100
LvP12	ATQGPLSTTL LDFWRMIWEY SVLIIVMACM EYEMGKKKCE RYWAEPGEMQ	150
PEP	ATQGPLSTTL LDFWRMIWEY RILVIVMACM EFEMGKKKCE RYWAEPGETQ	150
Consensus	ATQGPLSTTL LDFWRMIWEY ..L.IVMACM E.EMGKKKCE RYWAEPGE.Q	150
LvP12	LEFGPFSVSC EAEKRKSDYI IRTLKVKFNS ETRTIYQFHY KNWPDHDVPS	200
PEP	LQFGPFSISC EAEKKKSDYK IRTLKAKFNN ETRIIYQFHY KNWPDHDVPS	200
Consensus	L.FGPFS.SC EAEK.KSDY. IRTLK.KFN. ETR.IYQFHY KNWPDHDVPS	200
LvP12	SIDPILELIW DVRCYQEDDS VPICIHCSAG CGRTGVICAI VDYTWMLLKD	250
PEP	SIDPILQLIW DMRCYQEDDC VPICIHCSAG CGRTGVICAV -DYTWMLLKD	249
Consensus	SIDPIL.LIW D.RCYQEDD. VPICIHCSAG CGRTGVICA. .DYTWMLLKD	250
LvP12	GIIPENFSVF SLIREMRTQR PSLVQTQEQY ELVYN AVLEL FKRQMDVIRD	300
PEP	GIIPKNFSVF NLIQEMRTQR PSLVQTQEQY ELVYS AVLEL FKRHM D VID	299
Consensus	GIIP.NFSVF .LI.EMRTQR PSLVQTQEQY ELVY.AVLEL FKR.MDVI.D	300
LvP12	KHSGTESQAK HCIPEKNHTL QADSYSPNLP KSTTKAAKMM NQQR---TKM	347
PEP	NHLGREIQAQ CSIPEQSLTV EADSCPLDLP KNAMRDVKT T NQHSKOGAEA	349
Consensus	.H.G.E.QA. ..IPE...T. .ADS....LP K.....K.. NQ.....	350
LvP12	EIKESS S FD F RTSEISAKEE LVLHPAKSST SFDFLELNYS FDKNADTTMK	397
PEP	ESTGGSS L GL RTSTMNAEEE LVLHSAKSSP SPNCLELNCG CNNKAVITRN	399
Consensus	E....SS... RTS...A.EE LVLH.AKSS. SF..LELN..A..T..	400

FIGURE 2

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LyP12	WQTKAFFIVG EPLQKHQSLD LGSLLFEGCS NSKPVNAAGR YFNSKVPITR	447
PEP	GQARASPVVG EPLQKYQSLD FGSMLFGSCP SALPINTADR YHNSKGPVKR	449
Consensus	.Q..A.P.VG EPLQK.QSLD .GS.LF..C. ...P.N.A.R Y.NSK.P..R	450
LyP12	TKSTPFELIQ QRETKEVDSK <u>ENFSY</u> LESQP HDS-CFVEMQ AQKVMHVSSA	496
PEP	TKSTPFELIQ QRKTNDLAVG DGFSCLESQ LHEHYSLELQ VQRVAHVSSA	499
Consensus	TKSTPFELIQ QR.T..... ..FS.LESQ. H.....E.Q .Q.V.HVSS.	500
LyP12	ELNYSLPYDS KHQIRNASNV KHHDSALGV YSYIPLVENP YFSSWPPSGT	546
PEP	ELNYSLPYDS -----DASCV PRHSPGALRV HLYTSLAEDP YFSSSPNSA	544
Consensus	ELNYSLP... ..AS.V ..H...AL.V ..Y..L.E.P YFSS.PP...	550
LyP12	SSKMSLDLPE KQDGTVPFSS LLPTSSTSLF SYNSHNSLS LNSPTNISSL	596
PEP	DSKMSFDLPE KQDGATSPGA LLPASSTTSF FYSNPHDSL V MNTLTSTFSP	594
Consensus	.SKMS.DLPE KQDG...P.. LLP.SST..F .Y.N.H.SL. .N..T..S..	600
LyP12	LNQESAVLAT APRIDDEIPP <u>PLPVRT</u> PESF IVVEEAGEFS PNVPKSLSSA	646
PEP	LNQETAVEAP SRRTDDEIPP PLPERTPESF IVVEEAGEFS PRVTESL--P	642
Consensus	LNQE.AV.A. ..R.DDEIPP PLP.RTPESF IVVEEAGE.S P.V..SL...	650
LyP12	VKVIGITSLE WGGTSEPKKF DDSVILRPSK SVKLRSPKSE LHQDRSSPPP	696
PEP	LVTFTGASPE CSGTSEMKS- HDSVGFTPSK NVKLRSPKSD RHQDGSP-PP	690
Consensus	..V..G.S.E ..GTSE.K.. .DSV...PSK .VKLRSPKS. .HQD.S..PP	700
LyP1	***** PLPERTLESF FLADEDCMQA QSIETYSTSY PDTMENSTSS KQTLKTPGKS	746
PEP	PLPERTLESF FLADEDCIQA QAVQTSSTSY PETTENSTSS KQTLRTPGKS	740
Consensus	PLPERTLESF FLADEDC.QA Q...T.STSY P.T.ENSTSS KQTL.TPGKS	750
LyP1	FTRSKSLKIL RNMKKSICNS <u>CPPNK</u> PAESV QSNSSSFLN FGFANRFSKP	796
PEP	FTRSKSLKIF RNMKKSVCNS SSPSKPTERV QPKNSSSFLN FGFGNRFSKP	790
Consensus	FTRSKSLKI. RNMKKS.CNS ..P.KP.E.V Q..NSSSFLN FGF.NRFSKP	800
LyP1	***** KGPRNPPPTW NI	808
PEP	KGPRNPPSAW NM	802
Consensus	KGPRNPP..W N.	

↓ LyP2
GKNFSW L 692

FIGURE 2 CONT'D

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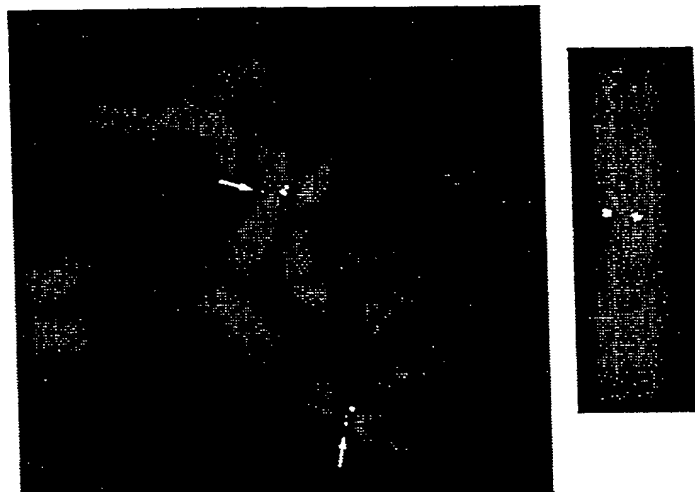


FIGURE 3A

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Chromosome 1

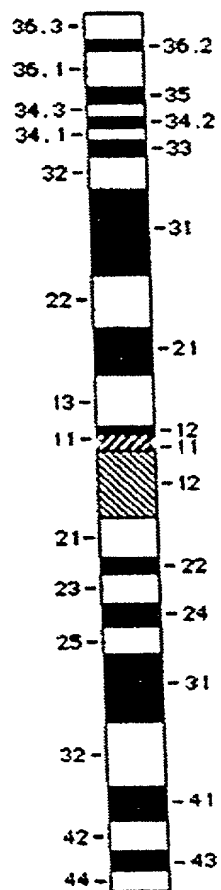


FIGURE 3B

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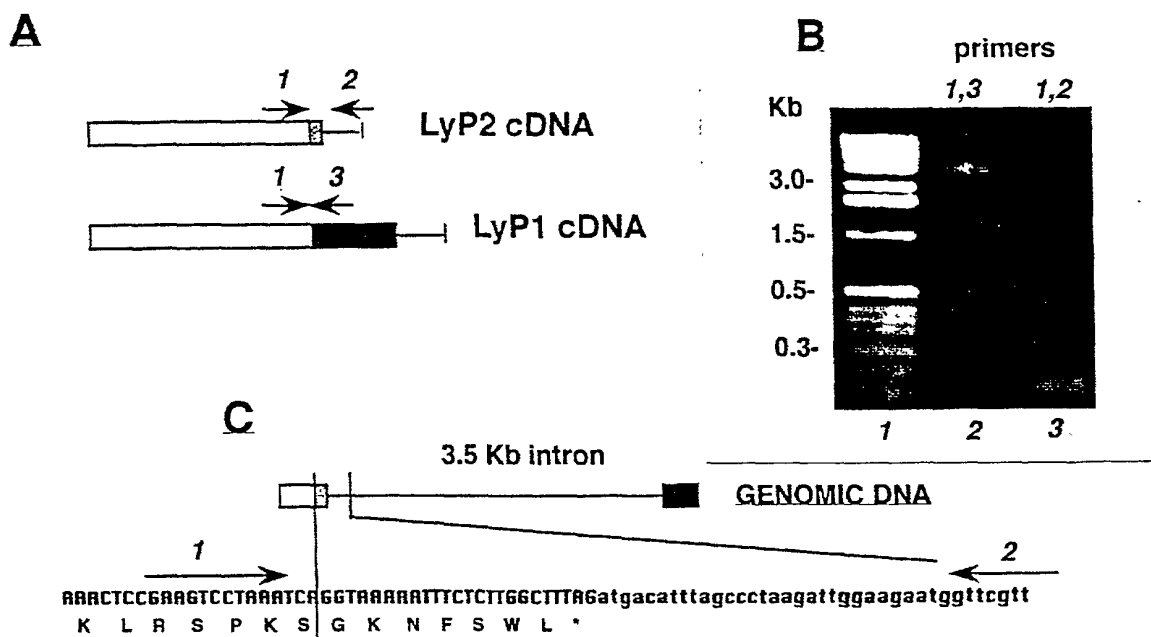


FIGURE 4

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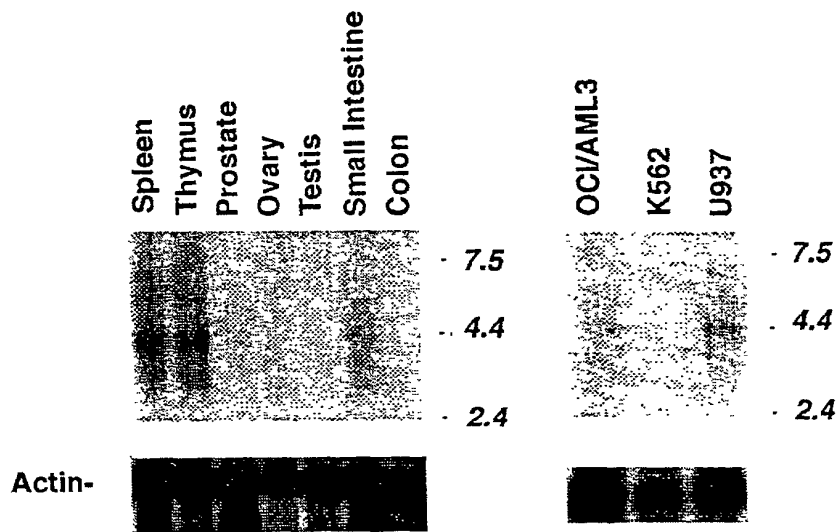


FIGURE 5A

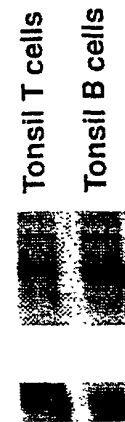


FIGURE 5B

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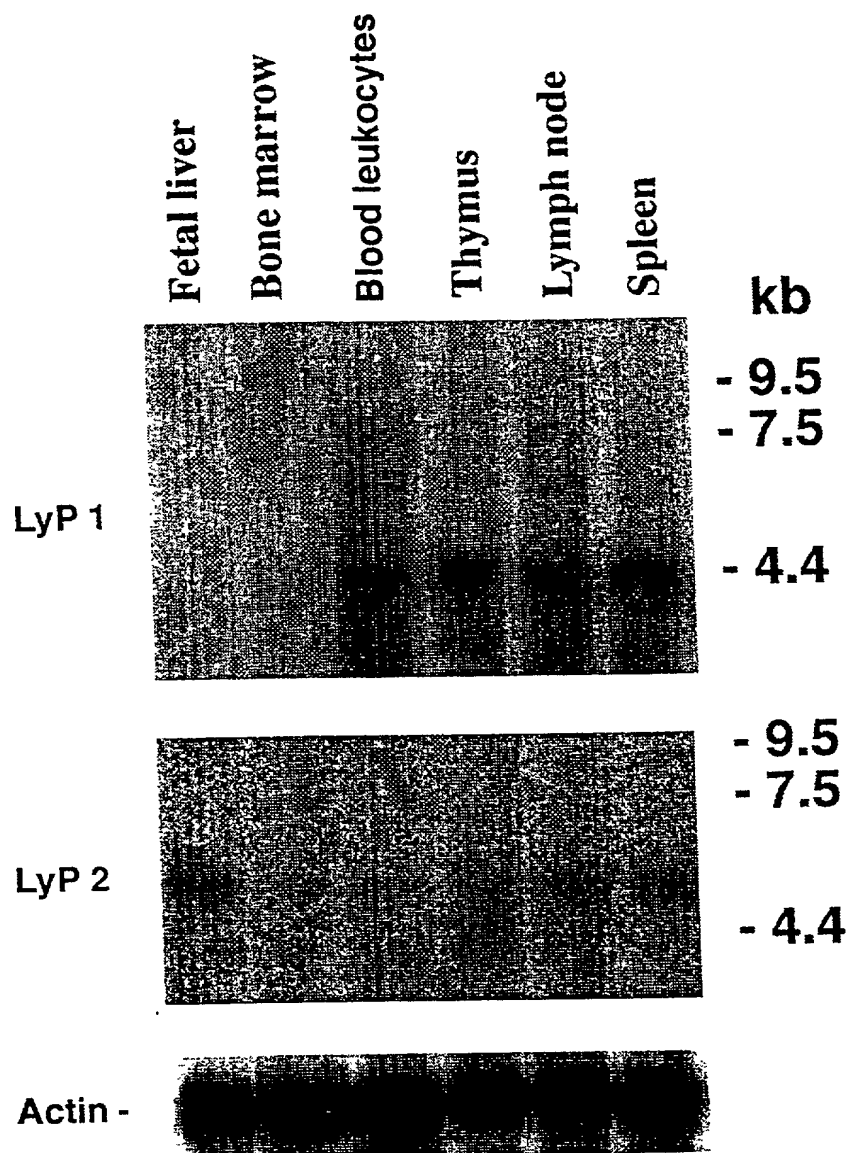


FIGURE 5C

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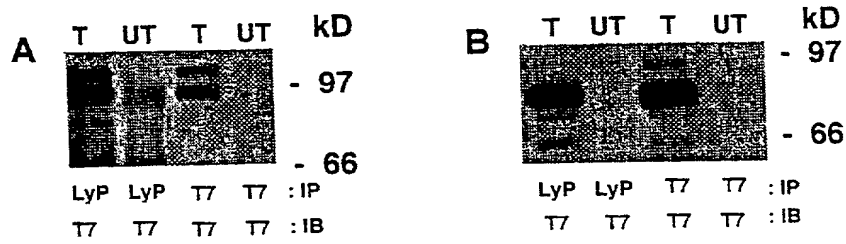


FIGURE 6

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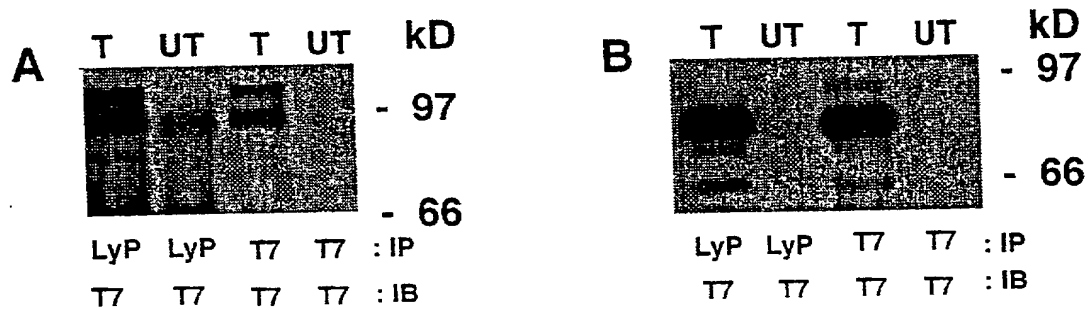


FIGURE 7

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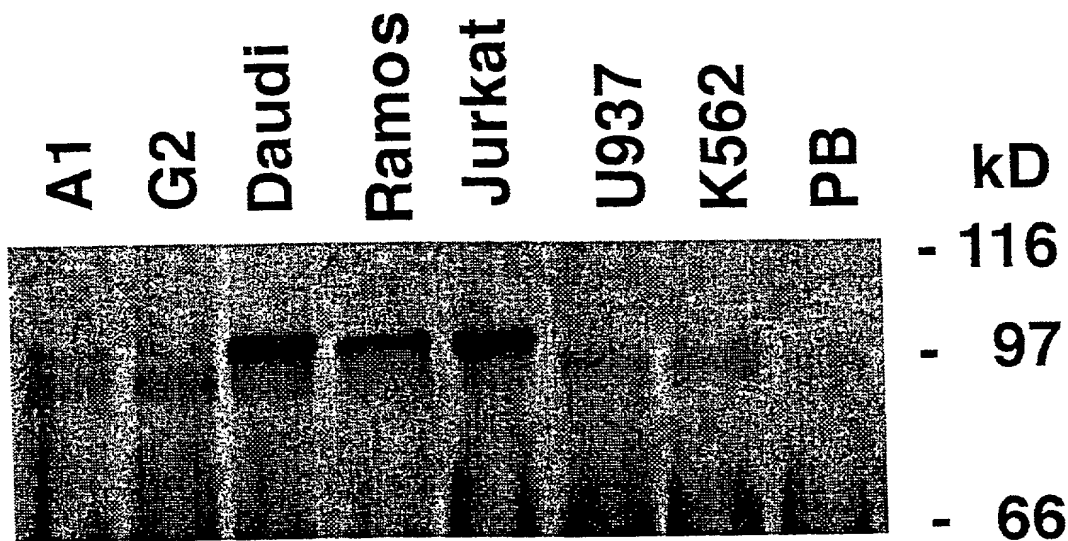


FIGURE 8

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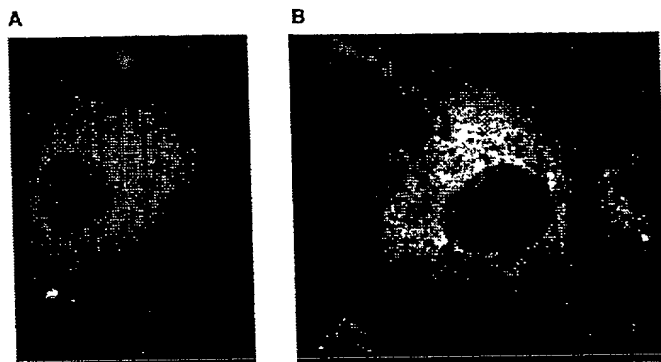


FIGURE 9

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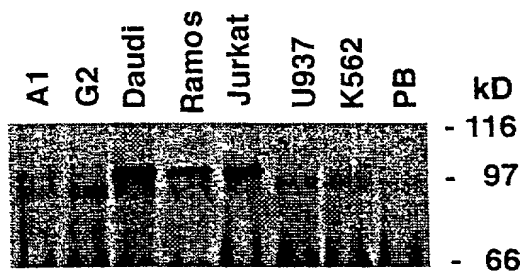


FIGURE 10

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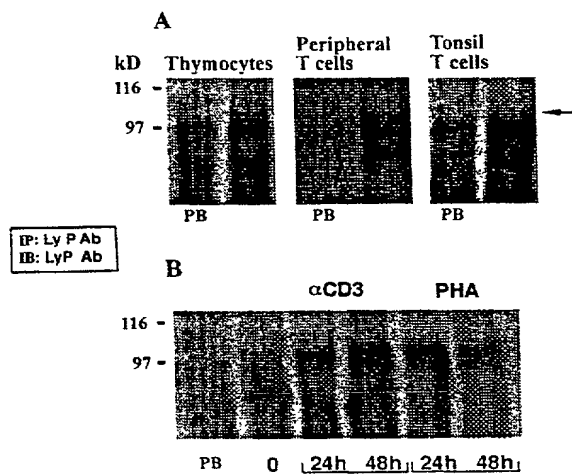


FIGURE 11

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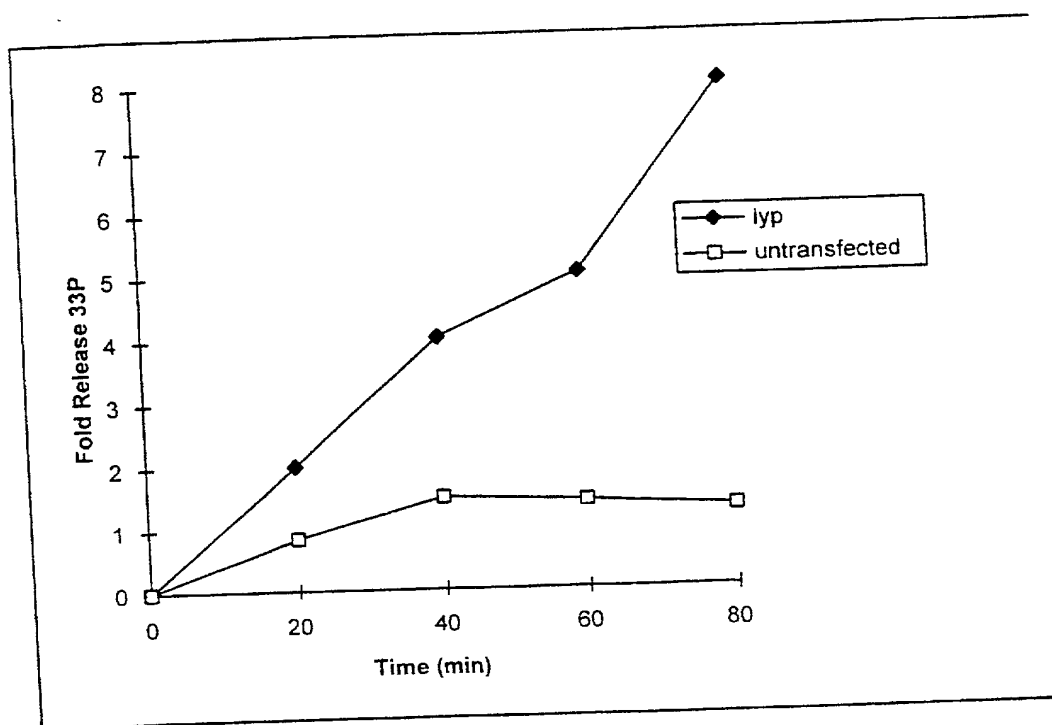


FIGURE 12

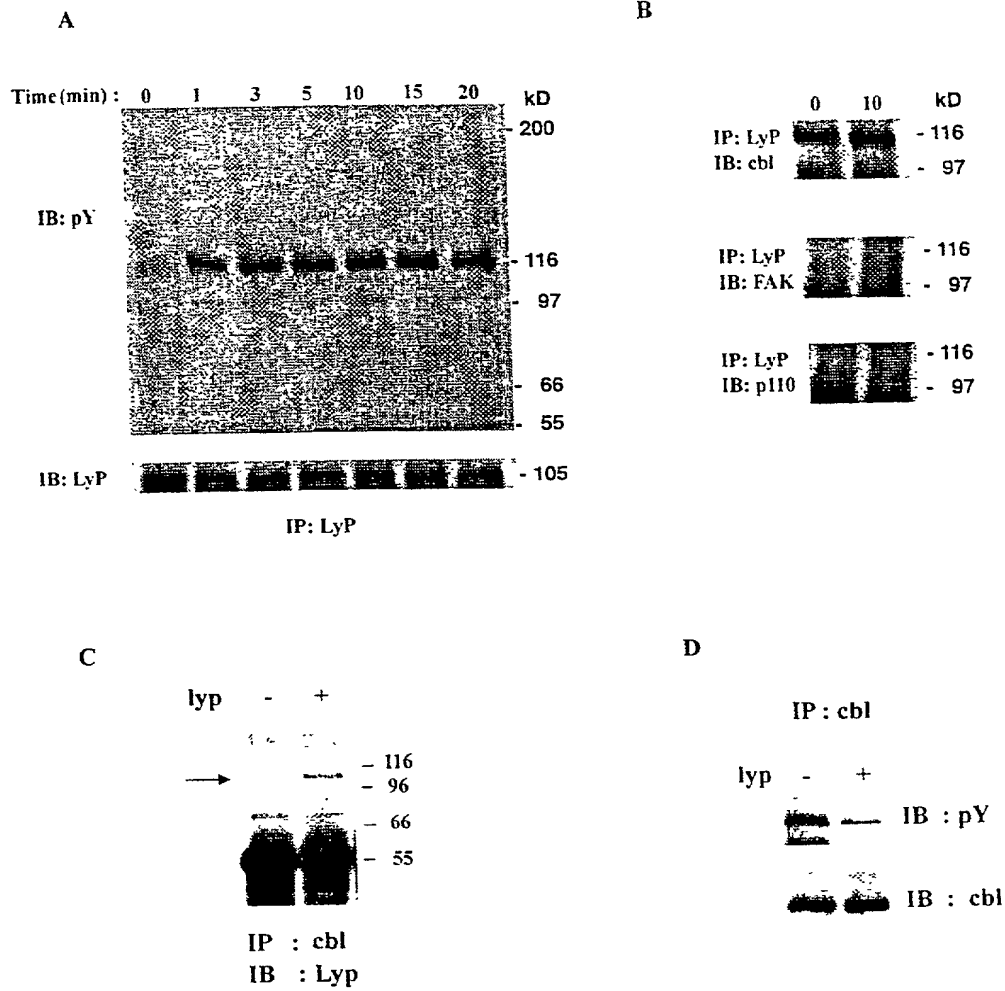


FIGURE 13

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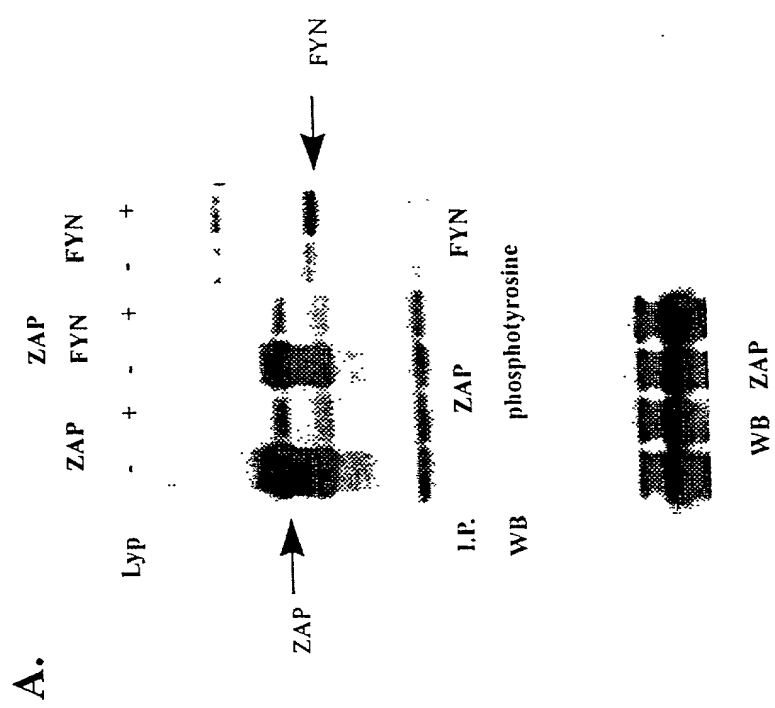
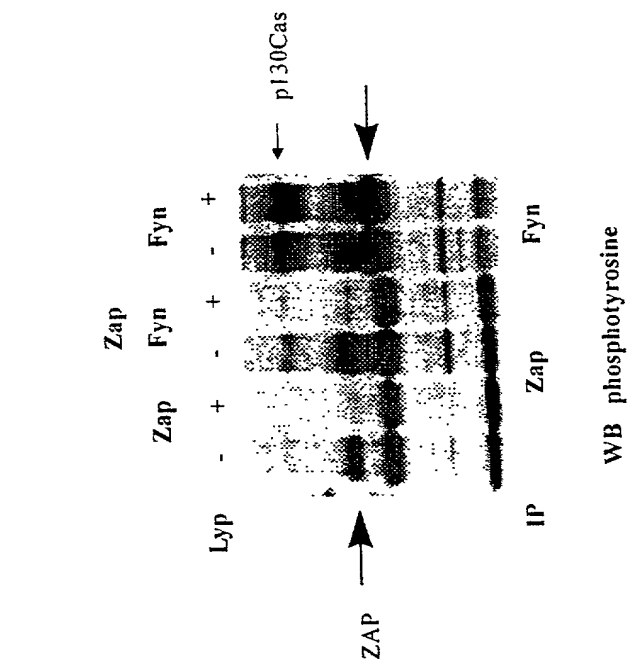
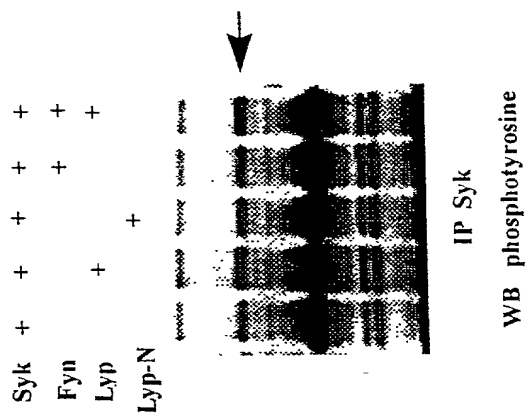


FIGURE 14

D.



C.

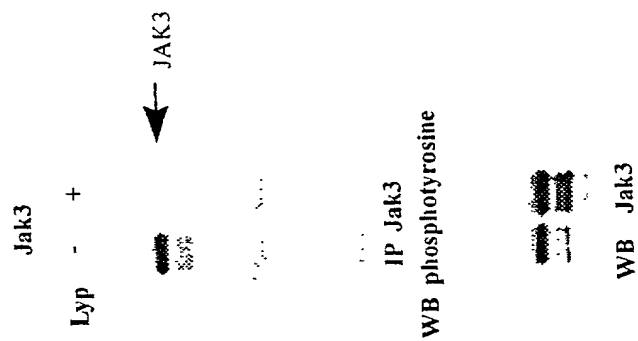


FIGURE 15

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

Attorney Docket No. 3477-88

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **HUMAN LYMPHOID PROTEIN TYROSINE PHOSPHATASES**,

the specification of which

☐ is attached hereto

OR

☒ was filed on January 18, 1999 as PCT International Application Number

PCT/CA99/00038 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or of any PCT International application having a filing date before that of the application on which priority is claimed.

2,220,853	Canada	01/16/1998	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Number	Country	MM/DD/YYYY Filed	Priority Claimed

			<input type="checkbox"/> Yes <input type="checkbox"/> No
Number	Country	MM/DD/YYYY Filed	Priority Claimed

			<input type="checkbox"/> Yes <input type="checkbox"/> No
Number	Country	MM/DD/YYYY Filed	Priority Claimed

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

Application Number(s)	Filing Date (MM/DD/YYYY)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application (37 C.F.R. § 1.63(d)).

Appln. Serial No.	Filing Date	Status Patented/Pending/Abandoned

Appln. Serial No.	Filing Date	Status Patented/Pending/Abandoned

Appln. Serial No.	Filing Date	Status Patented/Pending/Abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following registered attorney(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

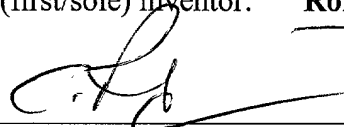
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Inventor's
Signature:  Date: Aug. 9, 2000

Residence: Toronto, Ontario, Canada

Citizenship: Canada

Post Office Address: 33 Christine Crescent
North York, Ontario M5G 1X8
Canada

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 Lys His Cys Ile Pro Glu Lys Asn His Thr Leu Gln Ala Asp Ser Tyr
 310 315 320 325

tct cct aat tta cca aaa agt acc aca aaa gca gca aaa atg atg aac 1064
 Ser Pro Asn Leu Pro Lys Ser Thr Thr Lys Ala Ala Lys Met Met Asn
 330 335 340

caa caa agg aca aaa atg gaa atc aaa gaa tct tct tcc ttt gac ttc 1112
 Gln Gln Arg Thr Lys Met Glu Ile Lys Glu Ser Ser Ser Phe Asp Phe
 345 350 355

agg act tct gaa ata agt gca aaa gaa gag cta gtt ttg cac cct gct 1160
 Arg Thr Ser Glu Ile Ser Ala Lys Glu Glu Leu Val Leu His Pro Ala
 360 365 370

aaa tca agc act tct ttt gac ttt ctg gag cta aat tac agt ttt gac 1208
 Lys Ser Ser Thr Ser Phe Asp Phe Leu Glu Leu Asn Tyr Ser Phe Asp
 375 380 385

aaa aat gct gac aca acc atg aaa tgg cag aca aag gca ttt cca ata 1256
 Lys Asn Ala Asp Thr Thr Met Lys Trp Gln Thr Lys Ala Phe Pro Ile
 390 395 400 405

gtt ggg gag cct ctt cag aag cat caa agt ttg gat ttg ggc tct ctt 1304
 Val Gly Glu Pro Leu Gln Lys His Gln Ser Leu Asp Leu Gly Ser Leu
 410 415 420

ttg ttt gag gga tgt tct aat tct aaa cct gta aat gca gca gga aga 1352
 Leu Phe Glu Gly Cys Ser Asn Ser Lys Pro Val Asn Ala Ala Gly Arg
 425 430 435

tat ttt aat tca aag gtg cca ata aca cgg acc aaa tca act cct ttt 1400
 Tyr Phe Asn Ser Lys Val Pro Ile Thr Arg Thr Lys Ser Thr Pro Phe
 440 445 450

gaa ttg ata cag cag aga gaa acc aag gag gtg gac agc aag gaa aac 1448
 Glu Leu Ile Gln Gln Arg Glu Thr Lys Glu Val Asp Ser Lys Glu Asn
 455 460 465

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ttt tct tat ttg gaa tct caa cca cat gat tct tgt ttt gta gag atg 1496
 Phe Ser Tyr Leu Glu Ser Gln Pro His Asp Ser Cys Phe Val Glu Met
 470 475 480 485

cag gct caa aaa gta atg cat gtt tct tca gca gaa ctg aat tat tca 1544
 Gln Ala Gln Lys Val Met His Val Ser Ser Ala Glu Leu Asn Tyr Ser
 490 495 500

ctg cca tat gac tct aaa cac caa ata cgt aat gcc tct aat gta aag 1592
 Leu Pro Tyr Asp Ser Lys His Gln Ile Arg Asn Ala Ser Asn Val Lys
 505 510 515

cac cat gac tct agt gct ctt ggt gta tat tct tac ata cct tta gtg 1640
 His His Asp Ser Ser Ala Leu Gly Val Tyr Ser Tyr Ile Pro Leu Val
 520 525 530

gaa aat cct tat ttt tca tca tgg cct cca agt ggt acc agt tct aag 1688
 Glu Asn Pro Tyr Phe Ser Ser Trp Pro Pro Ser Gly Thr Ser Ser Lys
 535 540 545

atg tct ctt gat tta cct gag aag caa gat gga act gtt ttt cct tct 1736
 Met Ser Leu Asp Leu Pro Glu Lys Gln Asp Gly Thr Val Phe Pro Ser
 550 555 560 565

tct ctg ttg cca aca tcc tct aca tcc ctc ttc tct tat tac aat tca 1784
 Ser Leu Leu Pro Thr Ser Ser Thr Ser Leu Phe Ser Tyr Tyr Asn Ser
 570 575 580

cat agt tct tta tca ctg aat tct cca acc aat att tcc tca cta ttg 1832
 His Ser Ser Leu Ser Leu Asn Ser Pro Thr Asn Ile Ser Ser Leu Leu
 585 590 595

aac cag gag tca gct gta cta gca act gct cca agg ata gat gat gaa 1880
 Asn Gln Glu Ser Ala Val Leu Ala Thr Ala Pro Arg Ile Asp Asp Glu
 600 605 610

atc ccc cct cca ctt cct gta cgg aca cct gaa tca ttt att gtg gtt 1928
 Ile Pro Pro Pro Leu Pro Val Arg Thr Pro Glu Ser Phe Ile Val Val
 615 620 625

gag gaa gct gga gaa ttc tca cca aat gtt ccc aaa tcc tta tcc tca 1976
 Glu Glu Ala Gly Glu Phe Ser Pro Asn Val Pro Lys Ser Leu Ser Ser
 630 635 640 645

gct gtg aag gta aaa att gga aca tca ctg gaa tgg ggt gga aca tct 2024
 Ala Val Lys Val Lys Ile Gly Thr Ser Leu Glu Trp Gly Gly Thr Ser
 650 655 660

5/20

gaa cca aag aaa ttt gat gac tct gtg ata ctt aga cca agc aag agt 2072
 Glu .Pro Lys Lys Phe Asp Asp Ser Val Ile Leu Arg Pro Ser Lys Ser
 665 670 675

gta aaa ctc cga agt cct aaa tca gaa cta cat caa gat cgt tct tct 2120
 Val Lys Leu Arg Ser Pro Lys Ser Glu Leu His Gln Asp Arg Ser Ser
 680 685 690

ccc cca cct cct ctc cca gaa aga act cta gag tcc ttc ttt ctt gcc 2168
 Pro Pro Pro Pro Leu Pro Glu Arg Thr Leu Glu Ser Phe Phe Leu Ala
 695 700 705

gat gaa gat tgt atg cag gcc caa tct ata gaa aca tat tct act agc 2216
 Asp Glu Asp Cys Met Gln Ala Gln Ser Ile Glu Thr Tyr Ser Thr Ser
 710 715 720 725

tat cct gac acc atg gaa aat tca aca tct tca aaa cag aca ctg aag 2264
 Tyr Pro Asp Thr Met Glu Asn Ser Thr Ser Ser Lys Gln Thr Leu Lys
 730 735 740

act cct gga aaa agt ttc aca agg agt aag agt ttg aaa att ttg cga 2312
 Thr Pro Gly Lys Ser Phe Thr Arg Ser Lys Ser Leu Lys Ile Leu Arg
 745 750 755

aac atg aaa aag agt atc tgt aat tct tgc cca cca aac aag cct gca 2360
 Asn Met Lys Lys Ser Ile Cys Asn Ser Cys Pro Pro Asn Lys Pro Ala
 760 765 770

gaa tct gtt cag tca aat aac tcc agc tca ttt ctg aat ttt ggt ttt 2408
 Glu Ser Val Gln Ser Asn Asn Ser Ser Ser Phe Leu Asn Phe Gly Phe
 775 780 785

gca aac cgt ttt tca aaa ccc aaa gga cca agg aat cca cca cca act 2456
 Ala Asn Arg Phe Ser Lys Pro Lys Gly Pro Arg Asn Pro Pro Pro Thr
 790 795 800 805

tgg aat att taataaaaact cagattttata ataatatggg ctgcaagtac 2505
 Trp Asn Ile

acctgcaaat aaaactacta gaatactgct agttaaaata agtgctctat atgcataata 2565

tgaagatatg ctaatgtgtt aatagctttt aaaagaaaag caaaatgccataaagtgcc 2625

gttttgcatt ttcatatcat ttgcattgag ttgaaaactg caaataaaaag tttgtcactt 2685

gagcttatgt acagaatgct atatgagaaa cacttttaga atggatttat ttttcatttt 2745

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tgccagttat ttttattttc ttttactttt ctacataaac ataaacttca aaaggtttgt 2805
 aagatttgga tctcaactaa tttctacatt gccagaatat actataaaaa gttaaaaaaa 2865
 aaaacttact ttgtgggttg caatacaaac tgctcttgac aatgactatt ccctgacagt 2925
 tatttttgcc taaatggagt ataccttgta aatcttccca aatgttgtgg aaaactggaa 2985
 tattaagaaa atgagaaatt atatttatta gaataaaaatg tgcaaataat gacaattatt 3045
 tgaatgtaac aag 3058

<210> 2

<211> 808

<212> PRT

<213> Homo sapiens

<400> 2

Met Asp Gln Arg Glu Ile Leu Gln Lys Phe Leu Asp Glu Ala Gln Ser
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Lys Lys Ile Thr Lys Glu Glu Phe Ala Asn Glu Phe Leu Lys Leu Lys
 20 25 30

Arg Gln Ser Thr Lys Tyr Lys Ala Asp Lys Thr Tyr Pro Thr Thr Val
 35 40 45

Ala Glu Asn Ala Lys Asn Ile Lys Lys Asn Arg Tyr Lys Asp Ile Leu
 50 55 60

Pro Tyr Asp Tyr Ser Arg Val Glu Leu Ser Leu Ile Thr Ser Asp Glu
 65 70 75 80

Asp Ser Ser Tyr Ile Asn Ala Asn Phe Ile Lys Gly Val Tyr Gly Pro
 85 90 95

Lys Ala Tyr Ile Ala Thr Gln Gly Pro Leu Ser Thr Thr Leu Leu Asp
 100 105 110

Phe Trp Arg Met Ile Trp Glu Tyr Ser Val Leu Ile Ile Val Met Ala
 115 120 125

Cys Met Glu Tyr Glu Met Gly Lys Lys Lys Cys Glu Arg Tyr Trp Ala
 130 135 140

Glu Pro Gly Glu Met Gln Leu Glu Phe Gly Pro Phe Ser Val Ser Cys
 145 150 155 160

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Glu Ala Glu Lys Arg Lys Ser Asp Tyr Ile Ile Arg Thr Leu Lys Val
 165 170 175

Lys Phe Asn Ser Glu Thr Arg Thr Ile Tyr Gln Phe His Tyr Lys Asn
 180 185 190

Trp Pro Asp His Asp Val Pro Ser Ser Ile Asp Pro Ile Leu Glu Leu
 195 200 205

Ile Trp Asp Val Arg Cys Tyr Gln Glu Asp Asp Ser Val Pro Ile Cys
 210 215 220

Ile His Cys Ser Ala Gly Cys Gly Arg Thr Gly Val Ile Cys Ala Ile
 225 230 235 240

Val Asp Tyr Thr Trp Met Leu Leu Lys Asp Gly Ile Ile Pro Glu Asn
 245 250 255

Phe Ser Val Phe Ser Leu Ile Arg Glu Met Arg Thr Gln Arg Pro Ser
 260 265 270

Leu Val Gln Thr Gln Glu Gln Tyr Glu Leu Val Tyr Asn Ala Val Leu
 275 280 285

Glu Leu Phe Lys Arg Gln Met Asp Val Ile Arg Asp Lys His Ser Gly
 290 295 300

Thr Glu Ser Gln Ala Lys His Cys Ile Pro Glu Lys Asn His Thr Leu
 305 310 315 320

Gln Ala Asp Ser Tyr Ser Pro Asn Leu Pro Lys Ser Thr Thr Lys Ala
 325 330 335

Ala Lys Met Met Asn Gln Gln Arg Thr Lys Met Glu Ile Lys Glu Ser
 340 345 350

Ser Ser Phe Asp Phe Arg Thr Ser Glu Ile Ser Ala Lys Glu Glu Leu
 355 360 365

Val Leu His Pro Ala Lys Ser Ser Thr Ser Phe Asp Phe Leu Glu Leu
 370 375 380

Asn Tyr Ser Phe Asp Lys Asn Ala Asp Thr Thr Met Lys Trp Gln Thr
 385 390 395 400

Lys Ala Phe Pro Ile Val Gly Glu Pro Leu Gln Lys His Gln Ser Leu
 405 410 415

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Asp Leu Gly Ser Leu Leu Phe Glu Gly Cys Ser Asn Ser Lys Pro Val
 420 425 430

Asn Ala Ala Gly Arg Tyr Phe Asn Ser Lys Val Pro Ile Thr Arg Thr
 435 440 445

Lys Ser Thr Pro Phe Glu Leu Ile Gln Gln Arg Glu Thr Lys Glu Val
 450 455 460

Asp Ser Lys Glu Asn Phe Ser Tyr Leu Glu Ser Gln Pro His Asp Ser
 465 470 475 480

Cys Phe Val Glu Met Gln Ala Gln Lys Val Met His Val Ser Ser Ala
 485 490 495

Glu Leu Asn Tyr Ser Leu Pro Tyr Asp Ser Lys His Gln Ile Arg Asn
 500 505 510

Ala Ser Asn Val Lys His His Asp Ser Ser Ala Leu Gly Val Tyr Ser
 515 520 525

Tyr Ile Pro Leu Val Glu Asn Pro Tyr Phe Ser Ser Trp Pro Pro Ser
 530 535 540

Gly Thr Ser Ser Lys Met Ser Leu Asp Leu Pro Glu Lys Gln Asp Gly
 545 550 555 560

Thr Val Phe Pro Ser Ser Leu Leu Pro Thr Ser Ser Thr Ser Leu Phe
 565 570 575

Ser Tyr Tyr Asn Ser His Ser Ser Leu Ser Leu Asn Ser Pro Thr Asn
 580 585 590

Ile Ser Ser Leu Leu Asn Gln Glu Ser Ala Val Leu Ala Thr Ala Pro
 595 600 605

Arg Ile Asp Asp Glu Ile Pro Pro Pro Leu Pro Val Arg Thr Pro Glu
 610 615 620

Ser Phe Ile Val Val Glu Glu Ala Gly Glu Phe Ser Pro Asn Val Pro
 625 630 635 640

Lys Ser Leu Ser Ser Ala Val Lys Val Lys Ile Gly Thr Ser Leu Glu
 645 650 655

Trp Gly Gly Thr Ser Glu Pro Lys Lys Phe Asp Asp Ser Val Ile Leu
 660 665 670

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Arg Pro Ser Lys Ser Val Lys Leu Arg Ser Pro Lys Ser Glu Leu His
675 680 685

Gln Asp Arg Ser Ser Pro Pro Pro Pro Leu Pro Glu Arg Thr Leu Glu
690 695 700

Ser Phe Phe Leu Ala Asp Glu Asp Cys Met Gln Ala Gln Ser Ile Glu
705 710 715 720

Thr Tyr Ser Thr Ser Tyr Pro Asp Thr Met Glu Asn Ser Thr Ser Ser
725 730 735

Lys Gln Thr Leu Lys Thr Pro Gly Lys Ser Phe Thr Arg Ser Lys Ser
740 745 750

Leu Lys Ile Leu Arg Asn Met Lys Lys Ser Ile Cys Asn Ser Cys Pro
755 760 765

Pro Asn Lys Pro Ala Glu Ser Val Gln Ser Asn Asn Ser Ser Ser Phe
770 775 780

Leu Asn Phe Gly Phe Ala Asn Arg Phe Ser Lys Pro Lys Gly Pro Arg
785 790 795 800

Asn Pro Pro Pro Thr Trp Asn Ile
805

<210> 3

<211> 2356

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (42)..(2117)

<400> 3

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Met Asp Gln Arg Glu
1 5

att ctg cag aag ttc ctg gat gag gcc caa agc aag aaa att act aaa 104
Ile Leu Gln Lys Phe Leu Asp Glu Ala Gln Ser Lys Lys Ile Thr Lys
10 15 20

gag gag ttt gcc aat gaa ttt ctg aag ctg aaa agg caa tct acc aag 152

10/20

Glu Glu Phe Ala Asn Glu Phe Leu Lys Leu Lys Arg Gln Ser Thr Lys
 25 30 35

tac aag gca gac aaa acc tat cct aca act gtg gct gag aat gcc aag 200
 Tyr Lys Ala Asp Lys Thr Tyr Pro Thr Thr Val Ala Glu Asn Ala Lys
 40 45 50

aat atc aag aaa aac aga tat aag gat att ttg ccc tat gat tat agc 248
 Asn Ile Lys Lys Asn Arg Tyr Lys Asp Ile Leu Pro Tyr Asp Tyr Ser
 55 60 65

cgg gta gaa cta tcc ctg ata acc tct gat gag gat tcc agc tac atc 296
 Arg Val Glu Leu Ser Leu Ile Thr Ser Asp Glu Asp Ser Ser Tyr Ile
 70 75 80 85

aat gcc aac ttc att aag gga gtt tat gga ccc aag gct tat att gcc 344
 Asn Ala Asn Phe Ile Lys Gly Val Tyr Gly Pro Lys Ala Tyr Ile Ala
 90 95 100

acc cag ggt cct tta tct aca acc ctc ctg gac ttc tgg agg atg att 392
 Thr Gln Gly Pro Leu Ser Thr Thr Leu Leu Asp Phe Trp Arg Met Ile
 105 110 115

tgg gaa tat agt gtc ctt atc att gtt atg gca tgc atg gag tat gaa 440
 Trp Glu Tyr Ser Val Leu Ile Ile Val Met Ala Cys Met Glu Tyr Glu
 120 125 130

atg gga aag aaa aag tgt gag cgc tac tgg gct gag cca gga gag atg 488
 Met Gly Lys Lys Lys Cys Glu Arg Tyr Trp Ala Glu Pro Gly Glu Met
 135 140 145

cag ctg gaa ttt ggc cct ttc tct gta tcc tgt gaa gct gaa aaa agg 536
 Gln Ieu Glu Phe Gly Pro Phe Ser Val Ser Cys Glu Ala Glu Lys Arg
 150 155 160 165

aaa tct gat tat ata atc agg act cta aaa gtt aag ttc aat agt gaa 584
 Lys Ser Asp Tyr Ile Ile Arg Thr Leu Lys Val Lys Phe Asn Ser Glu
 170 175 180

act cga act atc tac cag ttt cat tac aag aat tgg cca gac cat gat 632
 Thr Arg Thr Ile Tyr Gln Phe His Tyr Lys Asn Trp Pro Asp His Asp
 185 190 195

gta cct tca tct ata gac cct att ctt gag ctc atc tgg gat gta cgt 680
 Val Pro Ser Ser Ile Asp Pro Ile Leu Glu Leu Ile Trp Asp Val Arg
 200 205 210

tgt tac caa gag gat gac agt gtt ccc ata tgc att cac tgc agt gct 728

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Cys Tyr Gln Glu Asp Asp Ser Val Pro Ile Cys Ile His Cys Ser Ala
 215 220 225

ggc tgt gga agg act ggt gtt att tgt gct att gtt gat tat aca tgg 776
 Gly Cys Gly Arg Thr Gly Val Ile Cys Ala Ile Val Asp Tyr Thr Trp
 230 235 240 245

atg ttg cta aaa gat ggg ata att cct gag aac ttc agt gtt ttc agt 824
 Met Leu Leu Lys Asp Gly Ile Ile Pro Glu Asn Phe Ser Val Phe Ser
 250 255 260

ttg atc cgg gaa atg cgg aca cag agg cct tca tta gtt caa acg cag 872
 Leu Ile Arg Glu Met Arg Thr Gln Arg Pro Ser Leu Val Gln Thr Gln
 265 270 275

gaa caa tat gaa ctg gtc tac aat gct gta tta gaa cta ttt aag aga 920
 Glu Gln Tyr Glu Leu Val Tyr Asn Ala Val Leu Glu Leu Phe Lys Arg
 280 285 290

cag atg gat gtt atc aga gat aaa cat tct gga aca gag agt caa gca 968
 Gln Met Asp Val Ile Arg Asp Lys His Ser Gly Thr Glu Ser Gln Ala
 295 300 305

aag cat tgt att cct gag aaa aat cac act ctc caa gca gac tct tat 1016
 Lys His Cys Ile Pro Glu Lys Asn His Thr Leu Gln Ala Asp Ser Tyr
 310 315 320 325

tct cct aat tta cca aaa agt acc aca aaa gca gca aaa atg atg aac 1064
 Ser Pro Asn Leu Pro Lys Ser Thr Thr Lys Ala Ala Lys Met Met Asn
 330 335 340

caa caa agg aca aaa atg gaa atc aaa gaa tct tct tcc ttt gac ttt 1112
 Gln Gln Arg Thr Lys Met Glu Ile Lys Glu Ser Ser Ser Phe Asp Phe
 345 350 355

agg act tct gaa ata agt gca aaa gaa gag cta gtt ttg cac cct gct 1160
 Arg Thr Ser Glu Ile Ser Ala Lys Glu Glu Leu Val Leu His Pro Ala
 360 365 370

aaa tca agc act tct ttt gac ttt ctg gag cta aat tac agt ttt gac 1208
 Lys Ser Ser Thr Ser Phe Asp Phe Leu Glu Leu Asn Tyr Ser Phe Asp
 375 380 385

aaa aat gct gac aca acc atg aaa tgg cag aca aag gca ttt cca ata 1256
 Lys Asn Ala Asp Thr Thr Met Lys Trp Gln Thr Lys Ala Phe Pro Ile
 390 395 400 405

gtt ggg gag cct ctt cag aag cat caa agt ttg gat ttg ggc tct ctt 1304

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Val Gly Glu Pro Leu Gln Lys His Gln Ser Leu Asp Leu Gly Ser Leu	
410 415 420	
ttg ttt gag gga tgt tct aat tct aaa cct gta aat gca gca gga aga	1352
Leu Phe Glu Gly Cys Ser Asn Ser Lys Pro Val Asn Ala Ala Gly Arg	
425 430 435	
tat ttt aat tca aag gtg cca ata aca cgg acc aaa tca act cct ttt	1400
Tyr Phe Asn Ser Lys Val Pro Ile Thr Arg Thr Lys Ser Thr Pro Phe	
440 445 450	
gaa ttg ata cag cag aga gaa acc aag gag gtg gac agc aag gaa aac	1448
Glu Leu Ile Gln Gln Arg Glu Thr Lys Glu Val Asp Ser Lys Glu Asn	
455 460 465	
ttt tct tat ttg gaa tct caa cca cat gat tct tgt ttt gta gag atg	1496
Phe Ser Tyr Leu Glu Ser Gln Pro His Asp Ser Cys Phe Val Glu Met	
470 475 480 485	
cag gct caa aaa gta atg cat gtt tct tca gca gaa ctg aat tat tca	1544
Gln Ala Gln Lys Val Met His Val Ser Ser Ala Glu Leu Asn Tyr Ser	
490 495 500	
ctg cca tat gac tct aaa cac caa ata cgt aat gcc tct aat gta aag	1592
Leu Pro Tyr Asp Ser Lys His Gln Ile Arg Asn Ala Ser Asn Val Lys	
505 510 515	
cac cat gac tct agt gct ctt ggt gta tat tct tac ata cct tta gtg	1640
His His Asp Ser Ser Ala Leu Gly Val Tyr Ser Tyr Ile Pro Leu Val	
520 525 530	
gaa aat cct tat ttt tca tca tgg cct cca agt ggt acc agt tct aag	1688
Glu Asn Pro Tyr Phe Ser Ser Trp Pro Pro Ser Gly Thr Ser Ser Lys	
535 540 545	
atg tct ctt gat tta cct gag aag caa gat gga act gtt ttt cct tct	1736
Met Ser Leu Asp Leu Pro Glu Lys Gln Asp Gly Thr Val Phe Pro Ser	
550 555 560 565	
tct ctg ttg cca aca tcc tct aca tcc ctc ttc tct tat tac aat tca	1784
Ser Leu Leu Pro Thr Ser Ser Thr Ser Leu Phe Ser Tyr Tyr Asn Ser	
570 575 580	
cat agt tct tta tca ctg aat tct cca acc aat att tcc tca cta ttg	1832
His Ser Ser Leu Ser Leu Asn Ser Pro Thr Asn Ile Ser Ser Leu Leu	
585 590 595	
aac cag gag tca gct gta cta gca act gct cca agg ata gat gat gaa	1880

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Asn Gln Glu Ser Ala Val Leu Ala Thr Ala Pro Arg Ile Asp Asp Glu
 600 605 610

atc ccc cct cca ctt cct gta cgg aca cct gaa tca ttt att gtg gtt 1928
 Ile Pro Pro Pro Leu Pro Val Arg Thr Pro Glu Ser Phe Ile Val Val
 615 620 625

gag gaa gct gga gaa ttc tca cca aat gtt ccc aaa tcc tta tcc tca 1976
 Glu Glu Ala Gly Glu Phe Ser Pro Asn Val Pro Lys Ser Leu Ser Ser
 630 635 640 645

gct gtg aag gta aaa att gga aca tca ctg gaa tgg ggt gga aca tct 2024
 Ala Val Lys Val Lys Ile Gly Thr Ser Leu Glu Trp Gly Gly Thr Ser
 650 655 660

gaa cca aag aaa ttt gat gac tct gtg ata ctt aga cca agc aag agt 2072
 Glu Pro Lys Lys Phe Asp Asp Ser Val Ile Leu Arg Pro Ser Lys Ser
 665 670 675

gta aaa ctc cga agt cct aaa tca ggt aaa aat ttc tct tgg ctt 2117
 Val Lys Leu Arg Ser Pro Lys Ser Gly Lys Asn Phe Ser Trp Leu
 680 685 690

tagatgacat ttagccctaa gattggaaga atggttcggt aagtttagag taattcactt 2177

caggaagtta cttggttccc ataatagctt ccagtattca ttgatttatt tctggctttc 2237

ccagactaga aattttgtaa agagtcattgg gggaagctag ggctaaccag aaaataaaat 2297

aaaaataatg ggataaaaaa tcggaactac tgttttcccc ctagtcggag cacatccgg 2356

<210> 4

<211> 692

<212> PRT

<213> Homo sapiens

<400> 4

Met Asp Gln Arg Glu Ile Leu Gln Lys Phe Leu Asp Glu Ala Gln Ser
 1 5 10 15

Lys Lys Ile Thr Lys Glu Glu Phe Ala Asn Glu Phe Leu Lys Leu Lys
 20 25 30

Arg Gln Ser Thr Lys Tyr Lys Ala Asp Lys Thr Tyr Pro Thr Thr Val
 35 40 45

Ala Glu Asn Ala Lys Asn Ile Lys Lys Asn Arg Tyr Lys Asp Ile Leu

SUBSTITUTE SHEET (RULE 26)

305	310	315	320
Gln Ala Asp Ser Tyr Ser Pro Asn Leu Pro Lys Ser Thr Thr Lys Ala	325	330	335
Ala Lys Met Met Asn Gln Gln Arg Thr Lys Met Glu Ile Lys Glu Ser	340	345	350
Ser Ser Phe Asp Phe Arg Thr Ser Glu Ile Ser Ala Lys Glu Glu Leu	355	360	365
Val Leu His Pro Ala Lys Ser Ser Thr Ser Phe Asp Phe Leu Glu Leu	370	375	380
Asn Tyr Ser Phe Asp Lys Asn Ala Asp Thr Thr Met Lys Trp Gln Thr	385	390	400
Lys Ala Phe Pro Ile Val Gly Glu Pro Leu Gln Lys His Gln Ser Leu	405	410	415
Asp Leu Gly Ser Leu Leu Phe Glu Gly Cys Ser Asn Ser Lys Pro Val	420	425	430
Asn Ala Ala Gly Arg Tyr Phe Asn Ser Lys Val Pro Ile Thr Arg Thr	435	440	445
Lys Ser Thr Pro Phe Glu Leu Ile Gln Gln Arg Glu Thr Lys Glu Val	450	455	460
Asp Ser Lys Glu Asn Phe Ser Tyr Leu Glu Ser Gln Pro His Asp Ser	465	470	475
Cys Phe Val Glu Met Gln Ala Gln Lys Val Met His Val Ser Ser Ala	485	490	495
Glu Leu Asn Tyr Ser Leu Pro Tyr Asp Ser Lys His Gln Ile Arg Asn	500	505	510
Ala Ser Asn Val Lys His His Asp Ser Ser Ala Leu Gly Val Tyr Ser	515	520	525
Tyr Ile Pro Leu Val Glu Asn Pro Tyr Phe Ser Ser Trp Pro Pro Ser	530	535	540
Gly Thr Ser Ser Lys Met Ser Leu Asp Leu Pro Glu Lys Gln Asp Gly	545	550	555
Thr Val Phe Pro Ser Ser Leu Leu Pro Thr SerSer Thr Ser Leu Ph			560

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565 570 575
 Ser Tyr Tyr Asn Ser His Ser Ser Leu Ser Leu Asn Ser Pro Thr Asn
 580 585 590
 Ile Ser Ser Leu Leu Asn Gln Glu Ser Ala Val Leu Ala Thr Ala Pro
 595 600 605
 Arg Ile Asp Asp Glu Ile Pro Pro Pro Leu Pro Val Arg Thr Pro Glu
 610 615 620
 Ser Phe Ile Val Val Glu Glu Ala Gly Glu Phe Ser Pro Asn Val Pro
 625 630 635 640
 Lys Ser Leu Ser Ser Ala Val Lys Val Lys Ile Gly Thr Ser Leu Glu
 645 650 655
 Trp Gly Gly Thr Ser Glu Pro Lys Lys Phe Asp Asp Ser Val Ile Leu
 660 665 670
 Arg Pro Ser Lys Ser Val Lys Leu Arg Ser Pro Lys Ser Gly Lys Asn
 675 680 685
 Phe Ser Trp Leu
 690

 <210> 5
 <211> 802
 <212> PRT
 <213> Mus musculus

 <400> 5
 Met Asp Gln Arg Glu Ile Leu Gln Gln Leu Leu Lys Glu Ala Gln Lys
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 Lys Lys Leu Asn Ser Glu Glu Phe Ala Ser Glu Phe Leu Lys Leu Lys
 20 25 30
 Arg Gln Ser Thr Lys Tyr Lys Ala Asp Lys Ile Tyr Pro Thr Thr Val
 35 40 45
 Ala Gln Arg Pro Lys Asn Ile Lys Lys Asn Arg Tyr Lys Asp Ile Leu
 50 55 60
 Pro Tyr Asp His Ser Leu Val Glu Leu Ser Leu Leu Thr Ser Asp Glu
 65 70 75 80

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Asp Ser Ser Tyr Ile Asn Ala Ser Phe Ile Lys Gly Val Tyr Gly Pro
85 90 95

Lys Ala Tyr Ile Ala Thr Gln Gly Pro Leu Ser Thr Thr Leu Leu Asp
100 105 110

Phe Trp Arg Met Ile Trp Glu Tyr Arg Ile Leu Val Ile Val Met Ala
115 120 125

Cys Met Glu Phe Glu Met Gly Lys Lys Lys Cys Glu Arg Tyr Trp Ala
130 135 140

Glu Pro Gly Glu Thr Gln Leu Gln Phe Gly Pro Phe Ser Ile Ser Cys
145 150 155 160

Glu Ala Glu Lys Lys Lys Ser Asp Tyr Lys Ile Arg Thr Leu Lys Ala
165 170 175

Lys Phe Asn Asn Glu Thr Arg Ile Ile Tyr Gln Phe His Tyr Lys Asn
180 185 190

Trp Pro Asp His Asp Val Pro Ser Ser Ile Asp Pro Ile Leu Gln Leu
195 200 205

Ile Trp Asp Met Arg Cys Tyr Gln Glu Asp Asp Cys Val Pro Ile Cys
210 215 220

Ile His Cys Ser Ala Gly Cys Gly Arg Thr Gly Val Ile Cys Ala Val
225 230 235 240

Asp Tyr Thr Trp Met Leu Leu Lys Asp Gly Ile Ile Pro Lys Asn Phe
245 250 255

Ser Val Phe Asn Leu Ile Gln Glu Met Arg Thr Gln Arg Pro Ser Leu
260 265 270

Val Gln Thr Gln Glu Gln Tyr Glu Leu Val Tyr Ser Ala Val Leu Glu
275 280 285

Leu Phe Lys Arg His Met Asp Val Ile Ser Asp Asn His Leu Gly Arg
290 295 300

Glu Ile Gln Ala Gln Cys Ser Ile Pro Glu Gln Ser Leu Thr Val Glu
305 310 315 320

Ala Asp Ser Cys Pro Leu Asp Leu Pro Lys Asn Ala Met Arg Asp Val
325 330 335

18/20

Lys Thr Thr Asn Gln His Ser Lys Gln Gly Ala Glu Ala Glu Ser Thr
 340 345 350

Gly Gly Ser Ser Leu Gly Leu Arg Thr Ser Thr Met Asn Ala Glu Glu
 355 360 365

Glu Leu Val Leu His Ser Ala Lys Ser Ser Pro Ser Phe Asn Cys Leu
 370 375 380

Glu Leu Asn Cys Gly Cys Asn Asn Lys Ala Val Ile Thr Arg Asn Gly
 385 390 395 400

Gln Ala Arg Ala Ser Pro Val Val Gly Glu Pro Leu Gln Lys Tyr Gln
 405 410 415

Ser Leu Asp Phe Gly Ser Met Leu Phe Gly Ser Cys Pro Ser Ala Leu
 420 425 430

Pro Ile Asn Thr Ala Asp Arg Tyr His Asn Ser Lys Gly Pro Val Lys
 435 440 445

Arg Thr Lys Ser Thr Pro Phe Glu Leu Ile Gln Gln Arg Lys Thr Asn
 450 455 460

Asp Leu Ala Val Gly Asp Gly Phe Ser Cys Leu Glu Ser Gln Leu His
 465 470 475 480

Glu His Tyr Ser Leu Arg Glu Leu Gln Val Gln Arg Val Ala His Val
 485 490 495

Ser Ser Glu Glu Leu Asn Tyr Ser Leu Pro Gly Ala Cys Asp Ala Ser
 500 505 510

Cys Val Pro Arg His Ser Pro Gly Ala Leu Arg Val His Leu Tyr Thr
 515 520 525

Ser Leu Ala Glu Asp Pro Tyr Phe Ser Ser Ser Pro Pro Asn Ser Ala
 530 535 540

Asp Ser Lys Met Ser Phe Asp Leu Pro Glu Lys Gln Asp Gly Ala Thr
 545 550 555 560

Ser Pro Gly Ala Leu Leu Pro Ala Ser Ser Thr Thr Ser Phe Phe Tyr
 565 570 575

Ser Asn Pro His Asp Ser Leu Val Met Asn Thr Leu Thr Ser Phe Ser
 580 585 590

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Pro Pro Leu Asn Gln Glu Thr Ala Val Glu Ala Pro Ser Arg Arg Thr
595 600 605

Asp Asp Glu Ile Pro Pro Pro Leu Pro Glu Arg Thr Pro Glu Ser Phe
610 615 620

Ile Val Val Glu Glu Ala Gly Glu Pro Ser Pro Arg Val Thr Glu Ser
625 630 635 640

Leu Pro Leu Val Val Thr Phe Gly Ala Ser Pro Glu Cys Ser Gly Thr
645 650 655

Ser Glu Met Lys Ser His Asp Ser Val Gly Phe Thr Pro Ser Lys Asn
660 665 670

Val Lys Leu Arg Ser Pro Lys Ser Asp Arg His Gln Asp Gly Ser Pro
675 680 685

Pro Pro Pro Leu Pro Glu Arg Thr Leu Glu Ser Phe Phe Leu Ala Asp
690 695 700

Glu Asp Cys Ile Gln Ala Gln Ala Val Gln Thr Ser Ser Thr Ser Tyr
705 710 715 720

Pro Glu Thr Thr Glu Asn Ser Thr Ser Ser Lys Gln Thr Leu Arg Thr
725 730 735

Pro Gly Lys Ser Phe Thr Arg Ser Lys Ser Leu Lys Ile Phe Arg Asn
740 745 750

Met Lys Lys Ser Val Cys Asn Ser Ser Ser Pro Ser Lys Pro Thr Glu
755 760 765

Arg Val Gln Pro Lys Asn Ser Ser Ser Phe Leu Asn Phe Gly Phe Gly
770 775 780

Asn Arg Phe Ser Lys Pro Lys Gly Pro Arg Asn Pro Pro Ser Ala Trp
785 790 795 800

Asn Met

<210> 6

<211> 82

<212> DNA

<213> Homo sapiens

<400> 6

aagattggaa gaatggttcg tt

82

<211> 14

<213> Homo sapiens

Lys Leu Arg Ser Pro Lys Ser Gly Lys Asn Phe Ser Trp Leu

1

5

10

Parameter	Value	Standard Error	z	P	95% CI
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Age	0.000	0.000	0.000	1.000	0.000, 0.000
Gender	0.000	0.000	0.000	1.000	0.000, 0.000
Education	0.000	0.000	0.000	1.000	0.000, 0.000
Income	0.000	0.000	0.000	1.000	0.000, 0.000
Health	0.000	0.000	0.000	1.000	0.000, 0.000
Marital	0.000	0.000	0.000	1.000	0.000, 0.000
Religion	0.000	0.000	0.000	1.000	0.000, 0.000
Occupation	0.000	0.000	0.000	1.000	0.000, 0.000
Residence	0.000	0.000	0.000	1.000	0.000, 0.000
Time	0.000	0.000	0.000	1.000	0.000, 0.000
Constant	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Adjusted	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Constant	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Adjusted	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Constant	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Adjusted	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Constant	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Adjusted	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Constant	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Adjusted	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Constant	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Adjusted	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Constant	0.000	0.000	0.000	1.000	0.000, 0.000
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Adjusted	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Constant	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Adjusted	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000
Constant	0.000	0.000	0.000	1.000	0.000, 0.000
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Adjusted	0.000	0.000	0.000	1.000	0.000, 0.000
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Constant	0.000	0.000	0.000	1.000	0.000, 0.000
Model	0.000	0.000	0.000	1.000	0.000, 0.000